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[Volume 11

A STUDY OF THE VOLUMETRIC METHODS FOR THE ESTIMATION OF SMALL QUANTITIES OF FERROUS IRON IN THE PRESENCE OF ETHYL ALCOHOL AND ACETALDEHYDE

BY C. R. TALPADE

CHEMICAL LABORATORIES, ROYAL INSTITUTE OF SCIENCE, BOMBAY

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SUMMARY

An attempt has been made in this investigation to determine the accuracy of various methods which have been and can be employed for the determination of small amounts of ferrous iron formed in a solution of ferric salt in the presence of ethyl alcohol and acetaldehyde due to photo-chemical action. The titanous chloride method in which the amount of the unreduced ferric ions is estimated to deduce the amount of ferrous iron formed, is found to be accurate enough, but not practicable for the photo-reduction of ferric chloride. The dichromate method with diphenylamine as an internal indicator has been found to be quite unsuitable for this purpose, as a definite correction for the presence of alcohol and aldehyde cannot be applied. The ceric sulphate method using diphenylamine as an internal indicator is found to be the best method and preferable to the other two methods.

In the study of the chemical action of light on ferric chloride one has to estimate the amount of reduction that takes place in a given time. Under the circumstances of the photo-chemical experiment there are present in the reaction mixture organic substances such as alcohols or aldehydes, in addition to ferrous and ferric iron. Several methods are known for the volumetric estimation of iron. Some of these can be used when iron is present in the ferric state while others can be

employed when the solution contains iron in the ferrous condition only. An attempt has been made in this investigation to examine the accuracy of the various methods which have been and can be employed for the determination of the amount of ferrous iron in the systems mentioned above.

(i) *Estimation of ferric iron by thiosulphate.*—Ghosh and Purukayastha (*J. Indian Chem. Soc.*, 1929, **6**, 827) estimated the amount of unreduced ferric ions and thereby the amount of ferrous ions formed, by adding excess of sodium thiosulphate solution to the exposed solution and then titrating back the excess of thiosulphate against a standard solution of iodine using starch as an indicator. The same method was employed by Prasad and Sohoni (*J. Indian Chem. Soc.*, 1931, **8**, 489, 497) and Nigudkar (Thesis, Bombay University) for estimating the amount of unreduced ferric ions in an alcoholic solution of ferric chloride exposed to light. Limaye (*J. Indian Chem. Soc.*, 1933, **10**, 91, 101) compared this method with the dichromate method and found that lower results are obtained when thiosulphate method is used. Jellinek (*Zeit. anorg. Chem.*, 1924, 138, 79) has also pointed out this discrepancy and has suggested that the accuracy of the thiosulphate method may be increased if the titrations are carried out at 60°–70°.

(ii) *Estimation of ferric iron by titanous chloride.*—Lal and Gangully (*Zeit. anorg. Chem.*, 1936, 229) also employed the indirect method of estimating the amount of the unreduced ferric ions by the titanous chloride method in which ammonium thiocyanate or methylene blue is used as an internal indicator. The end point is indicated by the disappearance of the blood red colour in the former case and of the blue colour in the latter. It is found by the author that it is better to use ammonium thiocyanate than methylene blue because the reduced leuco-base of methylene blue rapidly re-oxidises. The re-oxidation is slow if the titrations are carried out in a current of carbon dioxide, but still a certain amount of re-oxidation does take place which makes the end point less sharp. No such difficulty arises in the use of ammonium thiocyanate which gives a sharp end point even in the presence of alcohol and acetaldehyde.

A number of estimations of solutions containing ferric iron were carried out when different quantities of ethyl alcohol, acetaldehyde and ferrous iron were added to them.

The method of preparing, storing and using titanous chloride was the one recommended by Knecht and Hibbert (New Reduction Methods in Volumetric Analysis, 1925 edition) But in spite of taking all the suggested precautions, titanous chloride slowly oxidises and its normality changes. Consequently it was standardised every time when used.

The results obtained when different amounts of alcohol are added to the same volume of the same solution of ferric alum are given in Table I.

TABLE I
0.0114 N TiCl_3

Ferric alum solution 0.025N	Alcohol added	TiCl_3 required	Correction
5 c.c.	0 c.c.	11.00 c.c.	+0.00 c.c.
5 "	1 "	10.95 "	+0.05 "
5 "	2 "	10.82 "	+0.18 "
5 "	3 "	10.90 "	+0.10 "
5 "	4 "	10.85 "	+0.15 "
5 "	5 "	11.05 "	-0.05 "
5 "	6 "	11.10 "	-0.10 "
5 "	7 "	10.88 "	+0.12 "
5 "	8 "	10.72 "	+0.28 "
5 "	9 "	11.00 "	0.00 "
5 "	10 "	11.45 "	-0.45 "

It will be seen from the above that the amount of correction is small and in some cases, negligible.

The results obtained when the same amount of ethyl alcohol is added to solutions containing different amounts of ferric alum are given in Table II.

TABLE II
0.0114 N TiCl_3

Ferric alum solution 0.025 N	Alcohol added	TiCl_3 required	TiCl_3 per c.c. of ferric alum solution	Correction
5 c.c.	0 c.c.	11.00 c.c.	2.20 c.c.	...
1 "	2 "	2.15 "	2.15 "	+0.05 c.c.
2 "	2 "	4.25 "	2.12 "	+0.15 "
3 "	2 "	6.45 "	2.15 "	+0.15 "
4 "	2 "	8.50 "	2.12 "	+0.30 "
5 "	2 "	10.75 "	2.12 "	+0.25 "
7 "	2 "	14.85 "	2.12 "	+0.55 "
8 "	2 "	16.95 "	2.12 "	+0.65 "
9 "	2 "	19.15 "	2.13 "	+0.65 "
10 "	2 "	21.50 "	2.15 "	+0.50 "

It will be seen from the fourth column that the volume of titanous chloride required to reduce the same amount of ferric iron in the presence of alcohol is practically the same, that is, the titre per c.c. is not appreciably affected by the increase in the amount of ferric alum in the solution. However, there is a definite effect of the addition of alcohol, the effect not varying uniformly with the volume of ferric alum solution taken for titration.

In order to examine the effect of ferrous iron on this method of estimation, mixtures containing the same amount of alcohol and having the same total iron content but different proportions of ferrous and ferric iron were employed. The results obtained are given in Table III. Ferrous ammonium sulphate solution was found to contain some ferric iron, 10 c.c. of the solution requiring 0.80 c.c. of the TiCl_3 solution. Correction for this has been made and the corrected readings are given in the 5th column of the following table.

TABLE III
0.0115 N TiCl_3

Ferric alum solution 0.025 N	Ferrous ammonium sulphate solution 0.025 N	Alcohol added	TiCl_3 required	Corrected readings	TiCl_3 per c.c. of ferric solution
5 c.c.	0 c.c.	0 c.c.	10.85 c.c.	10.85 c.c.	2.17 c.c.
0 "	10 "	0 "	0.80 "
1 "	9 "	2 "	2.80 "	2.08 "	2.08 "
2 "	8 "	2 "	5.05 "	4.41 "	2.20 "
3 "	7 "	2 "	7.30 "	6.74 "	2.25 "
4 "	6 "	2 "	9.45 "	8.97 "	2.24 "
5 "	5 "	2 "	11.40 "	11.00 "	2.20 "
6 "	4 "	2 "	13.55 "	13.23 "	2.20 "
7 "	3 "	2 "	15.70 "	15.46 "	2.21 "
8 "	2 "	2 "	17.70 "	17.54 "	2.19 "
9 "	1 "	2 "	19.80 "	19.72 "	2.19 "
10 "	0 "	2 "	21.60 "	21.60 "	2.16 "

These results again show that the titre per c.c. is not appreciably altered due to the presence of ferrous iron and alcohol.

Effect of the presence of acetaldehyde

Experiments similar to those described above were carried out in the presence of acetaldehyde instead of alcohol. The result obtained are given in the following tables. 2N solution of acetaldehyde was used for this purpose.

TABLE IV
0.0114 N TiCl_3

Ferric alum solution 0.025 N	Acetaldehyde solution added	TiCl_3 required	Correction
5 c.c.	0 c.c.	11.00 c.c.	..
5 "	1 "	11.05 "	-0.05 c.c.
5 "	2 "	11.10 "	-0.10 "
5 "	3 "	11.10 "	-0.10 "
5 "	4 "	11.10 "	-0.10 "
5 "	5 "	11.05 "	-0.05 "
5 "	6 "	11.10 "	-0.10 "
5 "	7 "	11.10 "	-0.10 "
5 "	8 "	11.10 "	-0.10 "
5 "	9 "	11.15 "	-0.15 "
5 "	10 "	11.25 "	-0.25 "

TABLE V
0.0107 N TiCl_3

Ferric alum solution 0.025N	Acetaldehyde solution added	TiCl_3 required	TiCl_3 per c.c. of ferric alum solution	Correction
5 c.c.	0 c.c.	11.65 c.c.	2.33 c.c.	...
1 "	2 "	2.32 "	2.32 "	+0.01 c.c.
2 "	2 "	4.70 "	2.35 "	-0.04 "
3 "	2 "	7.05 "	2.35 "	-0.06 "

Ferric alum solution 0.025N	Acetaldehyde solution added	TiCl ₃ required	TiCl ₃ per c.c. of ferric alum solution	Correction
4 c.c.	2 c.c.	9.40 c.c.	2.35 c.c.	-0.08 c.c.
5 "	2 "	11.45 "	2.29 "	+0.20 "
6 "	2 "	13.75 "	2.29 "	+0.23 "
7 "	2 "	16.30 "	2.33 "	+0.01 "
8 "	2 "	18.65 "	2.33 "	-0.01 "
9 "	2 "	20.90 "	2.32 "	+0.07 "
10 "	2 "	23.00 "	2.30 "	+0.30 "

TABLE VI
0.00962 N TiCl₃

Ferric alum solution 0.025 N	Ferrous ammonium sulphate solution 0.025 N	Acetaldehyde solution added	TiCl ₃ required	Corrected readings	TiCl ₃ per c.c. of ferric alum solution
5 c.c.	0 c.c.	0 c.c.	13.00 c.c.	13.00 c.c.	2.60 c.c.
0 "	10 "	0 "	1.15 "
1 "	9 "	2 "	3.65 "	2.61 "	2.61 "
2 "	8 "	2 "	6.25 "	5.33 "	2.66 "
3 "	7 "	2 "	8.65 "	7.84 "	2.61 "
4 "	6 "	2 "	11.25 "	10.56 "	2.64 "
5 "	5 "	2 "	13.75 "	13.17 "	2.63 "
6 "	4 "	2 "	16.10 "	15.64 "	2.61 "
7 "	3 "	2 "	18.30 "	17.95 "	2.56 "
8 "	2 "	2 "	20.70 "	20.47 "	2.61 "
9 "	1 "	2 "	23.25 "	23.13 "	2.57 "
10 "	0 "	2 "	25.45 "	25.45 "	2.54 "

These results lead to the conclusion that a small uncertain correction is necessary for the presence of alcohol, aldehyde and ferrous iron in the solution of ferric alum. This method may, however, be employed for the determination of the amount of photo-reduction in solutions of ferric chloride containing alcohol or acetaldehyde.

(iii) *Estimation of ferrous iron by potassium dichromate.*—The amount of ferrous iron present in these solutions is very small and, therefore, in order to determine the extent of reduction, it is more accurate to estimate the amount of ferrous iron directly, rather than to employ an indirect method. Consequently Limaye (loc. cit.) and Mohile (*Proc. Nat. Acad. Sci. India*, 1936, **6**, 261) used Knopp's method verified by Scott (*J. Amer. Chem. Soc.*, 1924, 1936) to estimate the amount of the ferrous iron in the partially reduced alcoholic solutions of ferric chloride as it has been found to yield satisfactory results in the presence of organic substances as well (cf. Scott: *Standard Methods of Analysis*). The method consists in titrating the solution containing ferrous ions against a solution of potassium dichromate using diphenylamine as an internal indicator. The end point indicated by the appearance of a blue colour is quite sharp and its advent can be judged by the development of the green colour due to the formation of chromic salts. The end point is made still sharper by the addition of a mixture containing hydrochloric and phosphoric acids which (i) removes the colour due to the ferric ions, (ii) increases the sensitivity of the indicator and (iii) lowers the oxidation potential of ferrous ions below the value at which the indicator shows the blue colour (cf. Schoenberger, *J. Amer. Chem. Soc.*, 1931, **83**, 30).

Limaye found that the concentrations of the acids and of the indicator recommended by Knopp do not give a sharp end point with solutions containing low concentration of ferrous ions. He employed the following solutions which are found to be more satisfactory.

(i) One part of concentrated HCl mixed with two parts of H_3PO_4 and the whole diluted to three times its volume. 10 c.c. of this mixture were used in each titration.

(ii) 0.008 M solution of diphenylamine in conc. H_2SO_4 . Two drops are used in each titration.

(iii) Total volume of the titrated solution was made up 50–60 c.c.

Limaye (loc. cit.) found however, that even with the above modifications in Knopp's method it was necessary to apply a correction to the titration readings for the presence of alcohol in the solutions. Mohile (loc. cit.) found it necessary to apply a correction to the titration readings for the presence of aldehyde also which is formed in alcoholic solution of ferric chloride exposed to light.

A systematic investigation was, therefore, undertaken to determine whether it is correct to make allowances in the titration readings in order to estimate the amount of ferrous ions in an alcoholic solution containing ferric ions and aldehyde by this

method. For this purpose different volumes of an aqueous solution of ferrous ammonium sulphate, acidified with dilute sulphuric acid, were titrated in the absence and presence of ethyl alcohol and acetaldehyde against a standard solution of $K_2Cr_2O_7$. The titrations were conducted in the same manner as carried out by Limaye. The results obtained when the same amount of ethyl alcohol is added to solutions containing different amounts of ferrous iron are given in Table VII

TABLE VII
0.025N $K_2Cr_2O_7$

Ferrous ammonium sulphate solution 0.04075 N	Alcohol added	$K_2Cr_2O_7$ required	$K_2Cr_2O_7$ per c.c. of ferrous ammonium sulphate solution	Correction
5 c.c.	0 c.c.	8.15 c.c.	1.63 c.c.	...
1 "	2 "	2.00 "	2.00 "	-0.37 c.c.
2 "	2 "	4.10 "	2.05 "	-0.84 "
3 "	2 "	6.20 "	2.06 "	-1.31 "
4 "	2 "	8.70 "	2.17 "	-2.18 "
5 "	2 "	10.20 "	2.04 "	-2.05 "
6 "	2 "	12.30 "	2.05 "	-2.52 "
7 "	2 "	14.05 "	2.01 "	-2.64 "
8 "	2 "	15.30 "	1.91 "	-2.26 "
9 "	2 "	17.80 "	1.98 "	-3.13 "
10 "	2 "	19.80 "	1.93 "	-3.50 "

It will be seen that the volume of $K_2Cr_2O_7$ solution required to oxidise the same volume of ferrous iron solution is greater in the presence of alcohol than when no alcohol is added. The titre per c.c. of ferrous ammonium sulphate for mixtures containing a fixed amount of alcohol and different amounts of ferrous iron is nearly but not exactly the same.

Later it was observed that more titre is required if the duration of titration is increased. The observed discrepancies in Tables VII and VIII may be due to this account, since the duration of titration was not kept constant

Table VIII gives the results obtained when different amounts of alcohol are added to the same volumes of the solution of ferrous ammonium sulphate,

TABLE VIII
0.025N $K_2Cr_2O_7$

Ferrous ammonium sulphate solution 0.04025 N	Alcohol added	$K_2Cr_2O_7$ required	Correction
5 c.c.	0.0 c.c.	8.05 c.c.	—
5 "	0.5 "	9.00 "	-0.95 c.c.
5 "	1.0 "	10.50 "	-2.45 "
5 "	1.5 "	10.95 "	-2.90 "
5 "	2.0 "	11.00 "	-2.95 "
5 "	3.0 "	11.55 "	-3.50 "
5 "	4.0 "	12.25 "	-4.20 "
5 "	5.0 "	12.95 "	-4.90 "

It will be seen from the above that the amount of correction increases from 0.95 to 4.9 as the amount of alcohol in the solution is increased from 0.5 c.c. to 5.0 c.c. This shows also that no fixed correction can be applied to the titration readings taken during the study of the photo-reduction of alcoholic solutions of ferric chloride as the amounts of both alcohol and ferrous ion are continuously changing with time.

In the above experiments iron only in the ferrous condition was examined the effect of the ferric iron, mixtures containing ferrous and ferric were employed. In each mixture the amount of ferrous iron is determined, the total quantity of iron and the amount of alcohol added are the same. The results obtained are given in Table IX.

TABLE IX
0.025N $K_2Cr_2O_7$

Ferrous ammonium solution 0.018N	Ferric chloride solution 0.018N	Alcohol added
10 c.c.	0 c.c.	0 c.c.
1 "	9 "	2 "
2 "	8 "	2 "
3 "	7 "	2 "
4 "	6 "	2 "

Ferrous ammonium solution 0.018N	Ferric chloride solution 0.018N	Alcohol added	$K_2Cr_2O_7$ required	$K_2Cr_2O_7$ per c.c. of ferrous ammo- nium sulphate solution
5 c.c.	5 c.c.	2 c.c.	5.40 c.c.	1.08 c.c.
6 "	4 "	2 "	6.75 "	1.12 "
7 "	3 "	2 "	7.60 "	1.08 "
8 "	2 "	2 "	6.95 "	0.87 "
9 "	1 "	2 "	9.75 "	1.08 "
10 "	0 "	2 "	10.20 "	1.02 "

These results again lead to the conclusion that the correction for the presence of alcohol is not a definite quantity.

EFFECT OF THE PRESENCE OF ACETALDEHYDE

Experiments similar to those described above were carried out in the presence of acetaldehyde (2N solution) instead of alcohol. The results obtained are given in Table X, XI and XII.

TABLE X
0.01 N $K_2Cr_2O_7$

Ferrous ammonium sulphate solution 0.0188N	Acetalde- hyde solu- tion added	$K_2Cr_2O_7$ required	$K_2Cr_2O_7$ per c.c. of ferrous ammonium sulphate solution	Correction
5 c.c.	0 c.c.	9.40 c.c.	1.88 c.c.	...
1 "	2 "	0.90 "	0.90 "	+0.98 c.c.
2 "	2 "	1.85 "	0.92 "	+1.91 "
3 "	2 "	4.50 "	1.50 "	+1.14 "
4 "	2 "	6.55 "	1.64 "	+0.99 "
5 "	2 "	8.15 "	1.63 "	+1.25 "
6 "	2 "	10.50 "	1.75 "	+0.78 "
7 "	2 "	12.25 "	1.75 "	+0.91 "
8 "	2 "	14.25 "	1.78 "	+0.79 "
9 "	2 "	15.45 "	1.72 "	+1.47 "
10 "	2 "	17.75 "	1.77 "	+1.05 "

TABLE XI
0.01N $K_2Cr_2O_7$

Ferrous ammonium sulphate solution 0.019 N	Acetaldehyde solution added	$K_2Cr_2O_7$ required	Correction
5 c.c.	0 c.c.	9.50 c.c.	0.00 c.c.
5 "	1 "	9.35 "	+0.15 "
5 "	2 "	9.20 "	+0.30 "
5 "	3 "	8.75 "	+0.75 "
5 "	4 "	8.15 "	+1.35 "
5 "	5 "	8.55 "	+0.95 "
5 "	6 "	7.15 "	+2.35 "
5 "	7 "	5.45 "	+4.05 "
5 "	8 "	5.10 "	+4.40 "
5 "	9 "	4.70 "	+4.80 "
5 "	10 "	4.40 "	+5.10 "

TABLE XII
0.01N $K_2Cr_2O_7$

Ferrous ammonium sulphate solution 0.018N	Ferric chloride solution 0.018N	Acetaldehyde solution added	$K_2Cr_2O_7$ required	$K_2Cr_2O_7$ per c.c. of ferrous ammonium sulphate solution
10 c.c.	0 c.c.	0 c.c.	18.15 c.c.	1.81 c.c.
1 "	9 "	2 "	0.10 "	0.10 "
2 "	8 "	2 "	0.80 "	0.40 "
3 "	7 "	2 "	3.00 "	1.00 "
4 "	6 "	2 "	5.60 "	1.40 "
5 "	5 "	2 "	8.25 "	1.65 "
6 "	4 "	2 "	10.65 "	1.77 "
7 "	3 "	2 "	12.00 "	1.71 "
8 "	2 "	2 "	14.00 "	1.75 "
9 "	1 "	2 "	15.90 "	1.77 "
10 "	0 "	2 "	17.50 "	1.75 "

These results lead to the same conclusion that the correction for the presence of aldehyde in a solution of ferric chloride containing varying amounts of ferrous ions and acetaldehyde is not a definite quantity. Further the correction required for the presence of acetaldehyde is opposite, though as large as that required for the presence of ethyl alcohol.

(iv) *Estimation of ferrous iron by ceric sulphate.*—This method which has been recently developed is an improvement on Knopp's method of estimating ferrous iron by dichromate. It consists in titrating a solution of ferrous iron with ceric sulphate in the presence of a suitable internal indicator. Some of the indicators which have been recommended by various investigators (Willard and Young, *J. Amer. Chem. Soc.*, 1928, **50**, 1322; 1334; 1368; 1372; 1379; Furman, *ibid.*, 755; 1676) are one per cent solutions of xylene cynol FF, methyl red, diphenylamine, diphenyl benzidine, tri-o-phenanthroline, etc

Ceric sulphate is a very convenient substitute in oxidimetric titrations since it forms a very stable solution and its reactions being concerned with the change from the quadrivalent to the tervalent stage, are more direct than those of permanganate in which the valency changes with the intermediate occurrence of other stages (*cf.* Ferman and Evans, *J. Amer. Chem. Soc.*, 1928, **50**, 1128). Further ceric salts are almost unique among the stable powerful oxidising agents in having a cation as the primary active constituent; in permanganate, dichromate, and others, the active constituent is an anion or at least can only be liberated by the destruction of an anion.

Willard and Young (*J. Amer. Chem. Soc.*, 1928, **50**, 1322) found that ceric sulphate can be used in a solution containing a high concentration of HCl and is stable towards heat. They showed that the normality of ceric sulphate solutions containing free sulphuric acid remains practically constant over a period of forty weeks and such a solution is not sensitive to light or air. They also (*J. Amer. Chem. Soc.*, 1928, **50**, 1334) found that diphenylamine and diphenyl benzidine are applicable as internal indicators for ferrous iron-ceric sulphate titrations just as they are applicable to ferrous iron-dichromate titrations. They found the colour change at the end point with ceric sulphate is much sharper than that obtained in the dichromate titration as the change from a green (chromic salt) colour to deep purple is much more difficult to recognise than that from a colourless solution to the deep purple shade. Comparing the effectiveness of the two indicators, diphenylamine and diphenyl benzidine, they found that diphenylamine is the better of the two as with the latter indicator a rather heavy white precipitate is formed which dissolves slowly whilst this does not happen with diphenylamine.

For the purpose of this investigation two drops of one per cent solution of diphenylamine in concentrated sulphuric acid were used as indicator in each

titration. A few c.c.s of syrupy phosphoric acid were added along with the indicator as this addition was found to make the end point very sharp

A number of estimations of solutions containing ferrous iron were made when different quantities of alcohol and acetaldehyde were added to them. The results obtained when different amounts of alcohol are added to the same volume of the same solution of ferrous ammonium sulphate are given in the following table.

TABLE XIII
0.0088 N Ceric sulphate.

Ferrous ammonium sulphate solution 0.017 N	Alcohol added	Ceric sulphate required	Correction
5 c.c.	0 c.c.	9.50 c.c.	0.00 c.c.
5 "	1 "	9.50 "	0.00 "
5 "	2 "	9.50 "	0.00 "
5 "	3 "	9.40 "	+0.10 "
5 "	4 "	9.50 "	0.00 "
5 "	5 "	9.30 "	+0.20 "
5 "	6 "	9.40 "	+0.10 "
5 "	7 "	9.30 "	+0.20 "
5 "	8 "	9.40 "	+0.10 "
5 "	9 "	9.30 "	+0.20 "
5 "	10 "	9.20 "	+0.30 "

It will be seen from the above table that practically no correction for the presence of alcohol is required in the titrations and the mean experimental error is not more than one per cent.

The results obtained when the same amount of ethyl alcohol is added to solutions containing different amounts of ferrous ammonium sulphate are given in Table XIV.

TABLE XIV
0.0088 N Ceric sulphate

Ferrous ammonium sulphate solution 0.017 N	alcohol added	Ceric sulphate required	Ceric sulphate per c.c. of ferrous ammonium sulphate solution	Correction
5 c.c.	0 c.c.	9.55 c.c.	1.91 c.c.	...
1 "	2 "	1.85 "	1.85 "	+0.06 c.c.
2 "	2 "	3.75 "	1.87 "	+0.07 "

Ferrous ammonium sulphate solution 0.017N	Alcohol added	Ceric sulphate required	Ceric sulphate per c.c. of ferrous ammonium sulphate solution	Correction
3 c.c.	2 c.c.	5.75 c.c.	1.92 c.c.	-0.02 c.c.
4 "	2 "	7.70 "	1.92 "	-0.06 "
5 "	2 "	9.55 "	1.91 "	0.00 "
6 "	2 "	11.40 "	1.90 "	+0.06 "
7 "	2 "	13.00 "	1.85 "	+0.37 "
8 "	2 "	15.15 "	1.89 "	+0.13 "
9 "	2 "	16.95 "	1.88 "	+0.24 "
10 "	2 "	18.85 "	1.88 "	+0.25 "

It will be seen from the last column that the volume of ceric sulphate required to oxidise the same amount of ferrous ammonium sulphate in the absence and presence of alcohol is the same within the experimental error. Also this volume is not affected by an increase in the amount of ferrous ammonium sulphate in the solution.

The effect of the presence of ferric iron on these titrations was examined by titrating mixtures containing the same amount of alcohol and having the same total iron content but different proportions of ferrous and ferric iron. The results are given in Table XV.

TABLE XV
0.0088N Ceric sulphate

Ferrous ammonium sulphate solution 0.016N	Ferric chlo- ride solution 0.016N	Alcohol added	Ceric sulphate required	Ceric sulphate per c.c. of ferrous ammonium sulphate solution
5 c.c.	0 c.c.	0 c.c.	9.30 c.c.	1.86 c.c.
1 "	9 "	2 "	1.85 "	1.85 "
2 "	8 "	2 "	3.65 "	1.82 "
3 "	7 "	2 "	5.50 "	1.83 "
4 "	6 "	2 "	7.40 "	1.85 "
5 "	5 "	2 "	9.20 "	1.84 "
6 "	4 "	2 "	11.05 "	1.84 "
7 "	3 "	2 "	12.90 "	1.84 "
8 "	2 "	2 "	14.75 "	1.84 "
9 "	1 "	2 "	16.60 "	1.84 "
10 "	0 "	2 "	18.45 "	1.84 "

It will be seen that the presence of ferric iron also does not affect the titrations. Moreover the accuracy in these titrations is very great.

EFFECT OF THE PRESENCE OF ACETALDEHYDE

Experiments similar to those described above were performed in the presence of acetaldehyde (2N solution) and the results obtained are given in the following tables.

TABLE XVI

0.0088N Ceric sulphate

Ferrous ammonium sulphate solution	Acetaldehyde solution added	Ceric sulphate required	Correction
5 c.c.	0 c.c.	8.90 c.c.	...
5 "	1 "	8.95 "	-0.05 c.c.
5 "	2 "	9.00 "	-0.10 "
5 "	3 "	9.00 "	-0.10 "
5 "	4 "	8.95 "	-0.05 "
5 "	5 "	8.95 "	-0.05 "
5 "	6 "	8.95 "	-0.05 "
5 "	7 "	9.00 "	-0.10 "
5 "	8 "	8.85 "	+0.05 "
5 "	9 "	8.95 "	-0.05 "
5 "	10 "	8.80 "	+0.10 "

TABLE XVII

0.0083N Ceric sulphate

Ferrous ammonium sulphate solution 0.017N	Acetaldehyde solution added 0.017N	Ceric sulphate required	Ceric sulphate per c.c. of ferrous ammonium sulphate solution	Correction
5 c.c.	0 c.c.	9.60 c.c.	1.92 c.c.	...
1 "	2 "	1.90 "	1.90 "	+0.02 c.c.
2 "	2 "	3.80 "	1.90 "	+0.04 "
3 "	2 "	5.70 "	1.90 "	+0.06 "
4 "	2 "	7.70 "	1.92 "	-0.02 "
5 "	2 "	9.50 "	1.90 "	+0.10 "

Ferrous ammonium sulphate solution 0.017N	Acetaldehyde solution added 0.017N	Ceric sulphate required	Ceric sulphate per c.c. of ferrous ammonium sulphate solution	Correction
6 c.c.	2 c.c.	11.30 c.c.	1.88 c.c.	+ 0.22 c.c.
7 "	2 "	13.20 "	1.88 "	+ 0.24 "
8 "	2 "	15.20 "	1.90 "	+ 0.16 "
9 "	2 "	16.90 "	1.88 "	+ 0.38 "
10 "	2 "	19.00 "	1.90 "	+ 0.20 "

TABLE XVIII

0.0088N Ceric sulphate

Ferrous ammonium sulphate solution 0.017N	Ferric chloride solution 0.017N	Acetaldehyde solution added	Ceric sulphate	Ceric sulphate per c.c. of ferrous ammonium sulphate solution
5 c.c.	0 c.c.	0 c.c.	9.55 c.c.	1.91 c.c.
1 "	9 "	2 "	1.90 "	1.90 "
2 "	8 "	2 "	3.80 "	1.90 "
3 "	7 "	2 "	5.80 "	1.93 "
4 "	6 "	2 "	7.60 "	1.90 "
5 "	5 "	2 "	9.25 "	1.85 "
6 "	4 "	2 "	11.20 "	1.87 "
7 "	3 "	2 "	13.20 "	1.88 "
8 "	2 "	2 "	14.85 "	1.85 "
9 "	1 "	2 "	16.50 "	1.85 "
10 "	0 "	2 "	18.65 "	1.86 "

It will be seen from the above that this method of titration is applicable to the estimation of ferrous iron in the presence of alcohol, acetaldehyde and ferric iron with the degree of accuracy which is obtained when these substances are not present. This method of estimation of ferrous iron can, therefore, be used in any mixture which contains any of these substances.

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TIME OF SETTING OF BARIUM MALONATE GELS AT DIFFERENT TEMPERATURES

BY KARTAR NARAIN MATHUR

CHEMICAL LABORATORIES, ROYAL INSTITUTE OF SCIENCE, BOMBAY

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SUMMARY

The time of setting of barium malonate gels prepared in methyl alcoholic solutions in the presence of glycerine has been measured at different temperatures with gel-forming mixtures containing different amounts of malonic acid. It has been found that generally with an increase in the amount of malonic acid the time of setting at first decreases rapidly, reaches a minimum value, then increases and reaches a maximum value and then decreases. It has been suggested that the observed changes in the setting time and in opacity of the gels are caused by changes in the hydrogen-ion concentration of the gel-forming mixtures.

Barium malonate gels were first prepared by Flade (*Zeit. anorg. Chem.*, 1913, **28**, 173) by mixing equivalent solutions in methyl alcohol of barium hydroxide and of malonic acid, in the presence of large amount of glycerine. Later Zocher and Albu (*Koll. Zeit.*, 1926, **46**, 33) studied the formation of these gels from mixtures containing (i) varying proportions of methyl alcohol and water and no glycerine, (ii) different proportions of glycerine and water and (iii) glycerine and different proportions of other alcohols. The gels obtained in the first and the second cases are generally turbid, the turbidity increasing with an increase in the proportion of water in the mixture. The gel formation starts earliest in mixture containing greatest quantity of water, the setting being delayed with the addition of increasing quantities of glycerine. The addition of an excess of malonic acid to the gel-forming mixture gives no gel while an increase in the amount of barium hydroxide gives rise to a snow-white pasty precipitate which is found to be very inhomogeneous.

Gels prepared with dehydrated glycerine are clear and transparent. They do not synerise and show normal double refraction and the phenomenon of thixotropy.

In the present investigation a systematic attempt has been made to determine the time of setting at various temperatures of barium malonate gels prepared in the presence of large amount of glycerine and a small quantity of water, using different amounts of barium hydroxide and malonic acid. When the constituents of the

gel-forming mixture—methyl alcoholic solutions of baryta and malonic acid—are mixed a precipitate is first obtained which disappears on shaking and gives rise to a homogeneous solution which sets to a firm gel. On keeping, the gels become opalescent without in any way altering their shape and structure.

EXPERIMENTAL

(a) *Preparation of solutions.*—Owing to the low solubility of barium hydroxide in methyl alcohol it is very difficult to prepare a solution of a definite concentration. It was found that a solution of the same concentration can be obtained any number of times if the procedure described below is precisely followed.

Barium hydroxide in sufficient excess is added to a litre of pure methyl alcohol, the mixture shaken for some time and then allowed to settle for 24 hours. The supernatant liquid is gently decanted and the excess of barium hydroxide, if any, is again allowed to settle till the solution is quite clear. This solution was stocked out of contact with air in such a manner that measured amounts of the solution can be taken out whenever required and was used throughout the investigation. The strength of this solution was found to be 0.235 N.

Solutions of malonic acid were prepared fresh for each experiment, because it was found by experience that the strength of the solution is considerably altered on keeping it for a long time.

Glycerine used in the preparation of gels was kept in a burette out of contact with air, and was taken out into a test tube fitted with a calcium chloride tube.

Redistilled water free from carbon dioxide was used in the preparation of gels.

(b) *Preparation of gels.*—4.0 c. c. of glycerine were taken out in a test tube and 1.0 c. c. of water was added to it. The tube was then thoroughly shaken to ensure complete mixing. 4.0 c. c. of baryta solution were then added to the mixture and the tube was again shaken till a homogeneous system was obtained (shaking is important in these experiments because glycerine does not mix easily with the other constituents). In another test tube a known amount of malonic acid solution of definite strength was taken and sufficient methyl alcohol was added to it so as to make up the total volume of the gel-forming mixture to 10 c. c. The two tubes with their contents were placed in a thermostat adjusted at a particular temperature for about 30 minutes during which time they acquired the temperature of the bath. The method of mixing given below was done throughout the investigation.

The tube containing the baryta solution was emptied into the tube containing the solution of malonic acid. The mixture was then transferred to the first tube

and this was repeated six times, as this much shaking was found to be sufficient and necessary to cause uniform mixing.

The tube containing the gel-forming mixture was then placed in the thermostat and the time of setting was determined by Fleming's method. The results obtained are given in the following tables in which the following symbols are used :—

Q=the quantity in c. c. of malonic acid solution in the mixture.

T=the time of setting in minutes.

The visual appearance of the gels is indicated by various marks mentioned below the table I. The absence of any mark indicates opaque gels.

TABLE I

Malonic acid solution = 1.0 N.

Q	Temp.=30°	Temp.=35°	Temp.=40°
0.05	No setting *	No setting *	No setting*
0.10	23.00 ‡	45.00 †	17.00 †
0.15	10.00 §	19.00 †	...
0.20	6.00 §	9.00 ‡	7.00 ‡
0.30	4.50	5.00 §	5.50 §
0.40	4.75	3.75 §	3.50
0.45	5.00
0.50	3.50	4.50 §	4.75
0.55	...	4.75 §	...
0.60	2.50	3.75 §	5.00
0.70	2.00	...	5.50

* Transparent solution.

† Transparent gel.

‡ Slightly turbid.

§ Turbid.

TABLE II

Malonic acid solution = 1.5 N.

Q	Temp. = 30°	Temp. = 35°	Temp. = 40°
0.05	After 2 hours †	After 2 hours †	52.00 †
0.10	11.50 †	15.00 †	14.00 †
0.20	4.50 §	5.50 §	6.00 §
0.30	3.50	4.00 §	4.50 §
0.35	4.00
0.40	3.00	4.50	5.50
0.45	3.00
0.50	2.50	3.50	6.50
0.60	2.25	3.00	4.00
0.70	2.20	2.50	2.75

TABLE III

Malonic acid solution = 2.0 N.

Q	Temp. = 30°	Temp. = 35°	Temp. = 40°
0.05	31.00 †	33.00 †	63.00 †
0.10	8.50 §	9.00 ‡	10.00 ‡
0.20	4.00	5.00 §	5.00 §
0.30	3.50	4.25	4.75
0.35	2.50
0.40	3.00	5.50	6.50
0.45	3.00	4.50	...
0.50	2.50	2.50	3.50
0.60	1.75	1.50	1.50
0.70	1.25	1.16	1.25
0.80	1.25

† Transparent gel.

‡ Slightly turbid.

§ Turbid.

TABLE IV

Malonic acid solution = 3.0 N.

Q	Temp. = 30°	Temp. = 35°	Temp. = 40°
0.05	11.00 ‡	18.00 ‡	29.00 ‡
0.10	5.50 §	6.50 §	7.00 §
0.20	4.00	4.75	5.75
0.25	4.00	4.00	8.00
0.30	3.00	4.75	4.75
0.40	1.50	1.75	2.25
0.50	Instantaneous	Instantaneous	1.50

It will be seen from the above tables that with an increase in the amount of malonic acid in the gel-forming mixture the time of setting of the gels at first decreases rapidly and then slowly until in all cases a minimum point is reached; it then increases slowly and reaches a maximum value after which it decreases continuously. This point has been clearly brought out in the curves (shown in Fig. 1) which refer to 1.5 N malonic acid. It will be seen from these curves that the rise after the minimum and the existence of the maximum are quite definite, as the several points which determine the nature of the curves are well distributed throughout.

These results are similar to those obtained in the case of silicic acid gels by Prasad and Hattiangadi (J. Indian Chem. Soc., 1929, **6**, 653) who observed that the time of setting of gels at first decreases, reaches a minimum value, then increases and reaches a maximum value and again decreases as the quantity of acidic ammonium acetate in the gel-forming mixture is increased. They explain their observation on the variation in the hydrogen ion concentration (cf. Prasad and Hattiangadi, J. Indian Chem. Soc., 1929, **6**, 893) which causes changes in the nature and the density of charge on the micelles in the gel-forming mixtures. The results obtained in this investigation cannot be explained in the same manner as above until the hydrogen ion concentrations of the various gel-forming mixtures are measured.

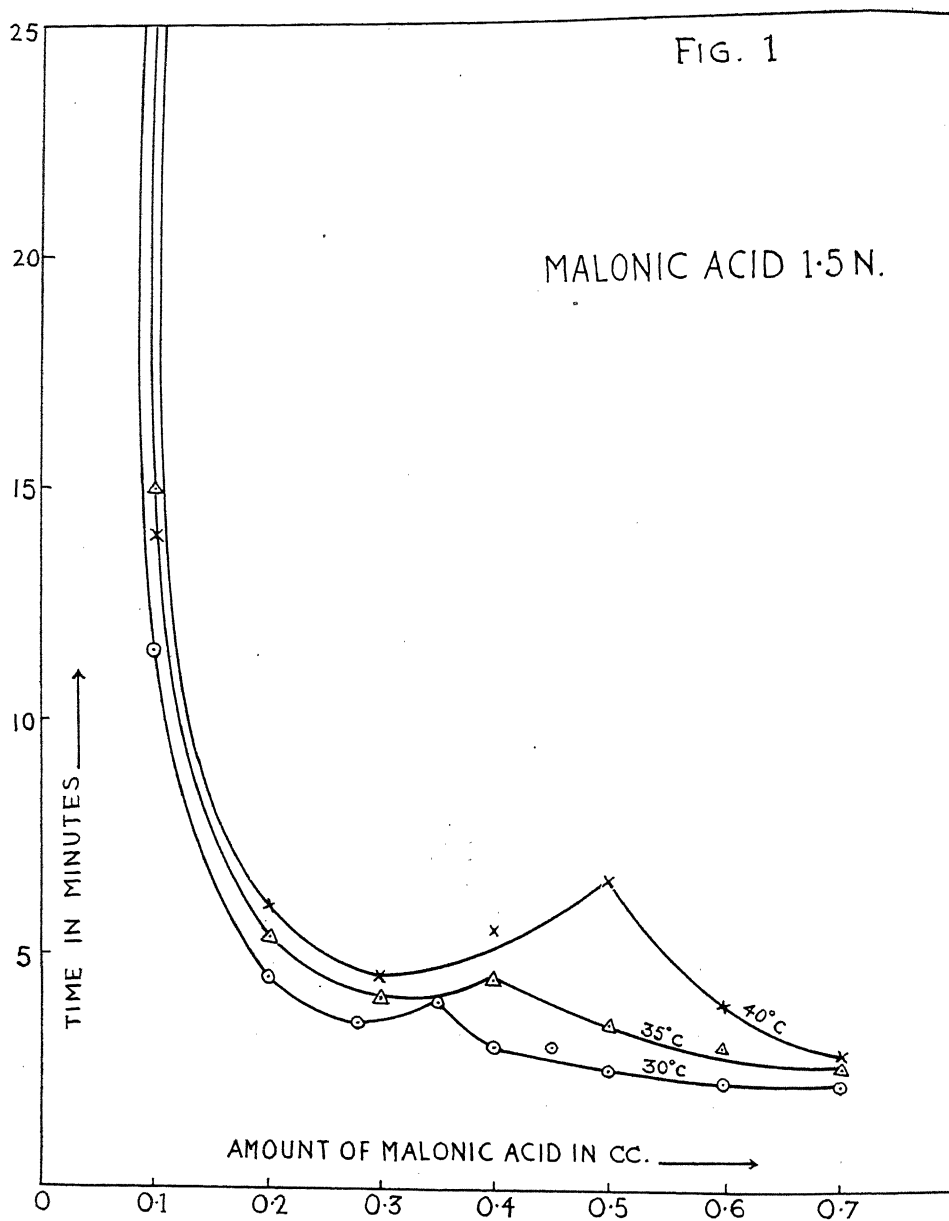
The barium malonate gels obtained in the presence of small quantities of malonic acid are formed apparently from negatively charged micelles. Since the amount of barium malonate and hence the number of micelles is small, the resulting gels are transparent. As the amount of malonic acid in the mixture is increased, the amount of barium malonate is also increased and the density of the negative charge on the micelles is decreased. The gels therefore appear translucent and set earlier. No explanation for the rise after the minimum and the subsequent fall after the maximum with a further increase in the amount of malonic acid in the gel-forming mixtures can be given at this stage.

The effect of temperature on the setting of barium malonate gels is not greatly marked but if comparisons are made under similar conditions, it is generally

‡ Slightly turbid.

§ Turbid.

observed that they set earlier at lower temperatures. This feature of barium malonate gels is similar to those of organic gels, but they cannot be placed in the same class as the organic gels because they are heat irreversible.



The author is thankful to Dr. Mata Prasad, D. Sc., F. I. C., for his suggestions and help.

A CONTRIBUTION TO THE STUDY OF THE GENUS *OPISTHORCHIS*
R. BLANCHARD 1895. PART I. DESCRIPTION OF NEW
SPECIES FROM BIRDS

BY RAM KRISHNA MEHRA

ZOOLOGY DEPARTMENT, UNIVERSITY OF ALLAHABAD

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SUMMARY

Four new species of the genus *Opisthorchis* R. Blanchard 1895 from the North Indian birds are described and their systematic position discussed.

The present paper deals with the description of four new species belonging to the genus *Opisthorchis* R. Blanchard 1895, which were obtained from a number of bird hosts examined during the last two years for helminthic collection

The first record of this genus from India, *O. noverca* Braun 1903 which is parasitic in man, pig, and dogs was recorded by Lewis and Cunningham in 1872. This species has been much discussed by various workers to define its limits whether it comprises two or only one species. Bhalariao (1931) while discussing the views of previous workers on this question from his investigations of a large amount of material collected by him comes to the conclusion that the forms included under it belong to two varieties. On the other hand Erhardt (1935) more recently in his comprehensive study of the genus in which he gives details of nearly all the species has decided to designate it as one species. Verma (1927) and Morgan (1927) added from India two species *O. pedicellata* Verma (1927) and *O. dendriticus* Morgan, 1927 from the fish and crane hosts respectively. Thapar in 1930 created a new genus *Gomtia* with the species *Gomtia piscicola* Thapar, 1930 from the siluroid fishes at Lucknow which as will be seen from the discussion in part II of this paper comes under the genus *Opisthorchis*. Lal in 1939 has added two more species *O. cheelis* and *O. giddhis* from the kite *Milvus migrans* and vulture *Sarcogyps calvus* from Lucknow. So in all about half a dozen species of this genus are known from India out of which *O. dendriticus* has been reduced to the rank of subspecies by Erhardt in 1935.

The work was carried out under the guidance of Dr. H. R. Mehra to whom I am much grateful for valuable help and advice. I am also thankful to the authorities of the Allahabad University for granting me a research scholarship and the laboratory facilities.

Opisthorchis allahabadii n. sp. (Fig. 1)

About a dozen specimens of this species were obtained from the gall bladder of Scavenger vulture *Sarcogyps calvus* obtained at Allahabad. All the specimens

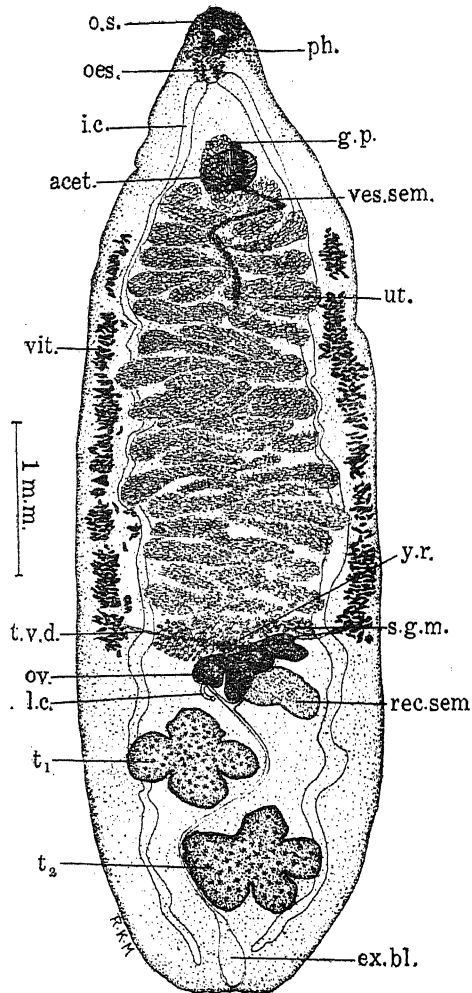


Fig. 1.—Dorsal view of *Opisthorchis allahabadii* n. sp.

acet., acetabulum; *c.e. bl.*, cornua of excretory bladder; *ex. bl.*, excretory bladder; *ex. p.*, excretory pore; *g.p.*, genital pore; *i.c.*, intestinal caecum; *l. c.*, Laurer's canal; *oes.*, oesophagus; *o. s.*, oral sucker; *ov.*, ovary; *ph.*, pharynx; *rec. sem.*, receptaculum seminis; *s. g. m.*, shell gland mass; *t1*, anterior testis; *t2*, posterior testis; *t. v. d.*, transverse vitelline duct; *ut.*, uterus; *vit.*, vitellaria; *ves. sem.*, vesicula seminalis; *y. r.*, yolk reservoir.

with me are in entire mounts. The body is muscular and greyish coloured anterior, posterior and lateral regions and in the median region dark yellow or sometimes brown due to closely packed mature ova. The body is pointed anteriorly and flat, roughly rounded at the posterior end measuring 5.5–6.6 mm. in length and 2.01 mm. in maximum breadth which lies in the region of the ovary. The breadth in the region of the acetabulum measures 1.24–1.425 mm. but behind the latter it is nearly uniform till the posterior extremity where it measures 0.51–0.825 mm. The bodywall is devoid of spines.

The oral sucker oval in shape 0.132×0.192 – 0.1815×0.21 mm. in size is sub-terminal facing ventrally. The prepharynx is entirely absent. The rounded pharynx is slightly larger than half the size of the oral sucker measuring 0.1221 mm. in diameter. The small bulb shaped oesophagus has 0.161 mm. length and 0.109 mm. maximum breadth near the intestinal bifurcation. The intestinal cæca after their origin run parallel to the body in a somewhat undulating manner, ending near the posterior extremity of the body a little in front of the excretory opening. The transversely elongated acetabulum of 0.264 – 0.29×0.2013 mm. size is thinner and less muscular than the oral sucker and the pharynx and lies at about one-sixth of the body length, *i.e.*, at 0.72–1.08 mm. from the anterior end. Around the oral sucker, pharynx and oesophagus are present certain elongated, narrow spindle shaped cells having the form of fine muscle fibres. These cells of probably glandular nature are characteristic of this species of the genus *Opisthorchis*.

The excretory pore lies terminally at the posterior end. The excretory bladder of an elongated S-shaped form passes between the two testes and bifurcates a little behind the median lobe of the ovary. The course of the two cornua in front of the ovary is not visible in entire mounts.

The ovary and testes are situated in the posterior one-third part of the body length. The testes are large, unequal, deeply lobed, similar in shape, intercæcal and obliquely situated. The anterior testis is always smaller and composed of four lobes, measuring 0.525–0.75 mm. in length and 0.6–0.9 mm. in maximum breadth. The four or five lobed posterior testis measures 0.5–0.75 mm. in length and 0.7–1.05 mm. in maximum breadth. The short narrow thin walled tubular vesicula seminalis lies to the right side twisted in two or three coils of 0.033–0.07 mm. maximum breadth, lies freely in the parenchyma commencing 0.63–0.75 mm. behind the acetabulum and opens into the genital atrium immediately in front of the ventral sucker. The cirrus sac and the prostate gland cells are absent.

The ovary measuring 0.15–0.345 mm in length and 0.615–0.87 mm. in maximum breadth has always a characteristic trifoliate form and is situated slightly to the right side, 1.425–1.89 mm. distance in front of the hinder end of the body. Each of the three parts of the ovary is divided again into two lobes. The large shell gland mass lies median closely in front of the ovary between the two intestinal

cæca. The receptaculum seminis, 0.675–0.8 mm. long and 0.225–0.285 mm. broad, is sac shaped and situated transversely with its proximal one-third part overlapped by the ovary. The Laurer's canal 0.0297–0.0462 mm. in diameter opens to the exterior in the region of the receptaculum seminis, behind the ovary. The uterus as it arises from the anterior end of the shell gland mass passes forwards in transversely and obliquely arranged coils within the intestinal cæca between the shell gland mass and the acetabulum, sometimes extending outside the cæca. The coils are, however, not much crowded together to form a compact mass. Terminally the uterus extends a little in front of the acetabulum to open into the genital atrium. The metraterm is absent. The ova are numerous, oval in shape, deep brown in colour when mature and measure 0.0231 mm. in length and 0.0132 mm. in maximum breadth.

The vitellaria lie laterally in the form of bands between the intestinal cæca and the body wall, commencing 0.375–0.525 mm. behind the acetabulum and terminating nearly at the same level in the region of the ovary. They are not clearly divided into ascini and in no case they have been found to extend behind the ovary. The transverse vitelline ducts arise from the posterior end of the vitellaria and unite in the median line dorsally to the shell gland complex to form the yolk reservoir situated just in front of the ovary.

Remarks:—*O. allahabadii* n. sp. resembles the other species of the genus in the extent of the intestinal cæca, shape of the excretory bladder, topography of the genital organs, arrangement of the uterine coils and in the position of the genital opening. But it differs from them in the size and shape of the body which is more or less leaf-shaped, in having the oral sucker, pharynx and oesophagus surrounded by the characteristic spindle-shaped gland cells, in the 1:2 ratio of the oral and the ventral suckers and in the characteristic shape of the ovary which though trifoliate as in *viverrini* (Poirier, 1886), *sinensis* (Cobb., 1875) *obsequens* (Nicoll, 1914), and *l. asiaticus* (Skrjabin, 1913) has each lobe divided again into two parts. It, however, bears some resemblance to *O. tenuicollis* (Rud. 1819) and specially the subspecies *O. tenuicollis. geminus* (Looss 1896) in having the lobed testes of a more or less similar shape, in the trilobed condition of the ovary, position of the shell gland mass just in front of the ovary and on account of the vitellaria which extend from the region of the ovary to some distance behind the ventral sucker. In the position of the ventral sucker, size of the pharynx which is half of the oral sucker, size of the oesophagus which is equal to that of the pharynx, and in the size of the ova it resembles *O. interruptus* Braun 1902.

Opisthorchis ahingii n. sp. (Fig. 2.) (a), (b), (c) and (d).

This species occurs very commonly in the biliary ducts of the common Indian darter *Ahinga melanogaster*. It is extremely difficult to obtain an entire

specimen on account of the long and coiled body occupying practically the whole of the space in the gall bladder. So far I have been able to get specimens cut into

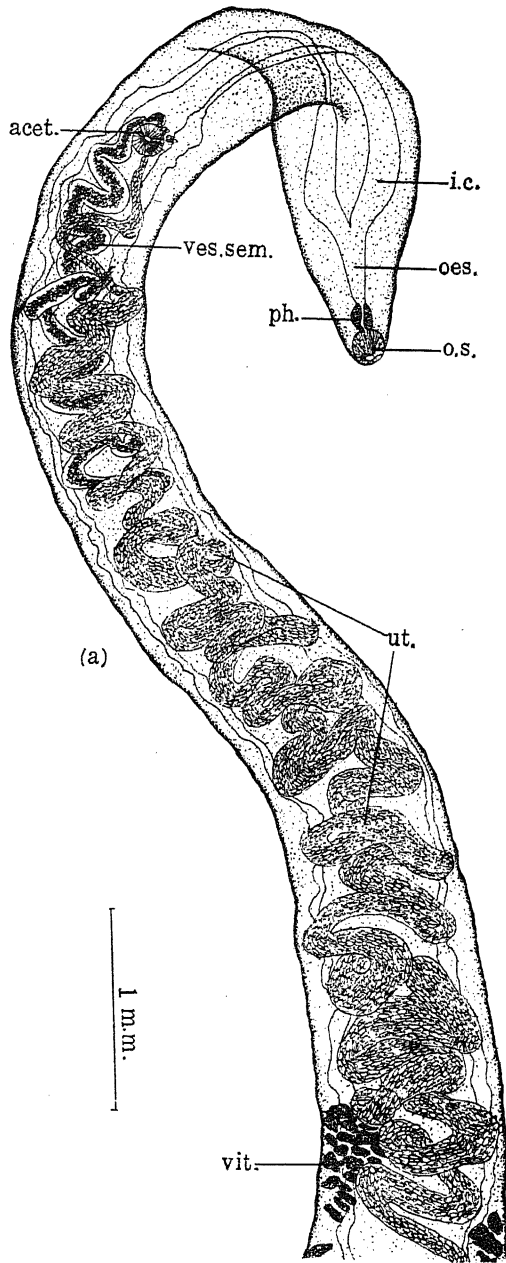


Fig. 2.—Ventral view of *Opisthorchis alvingii* n. sp.

(a) anterior portion upto vitellaria

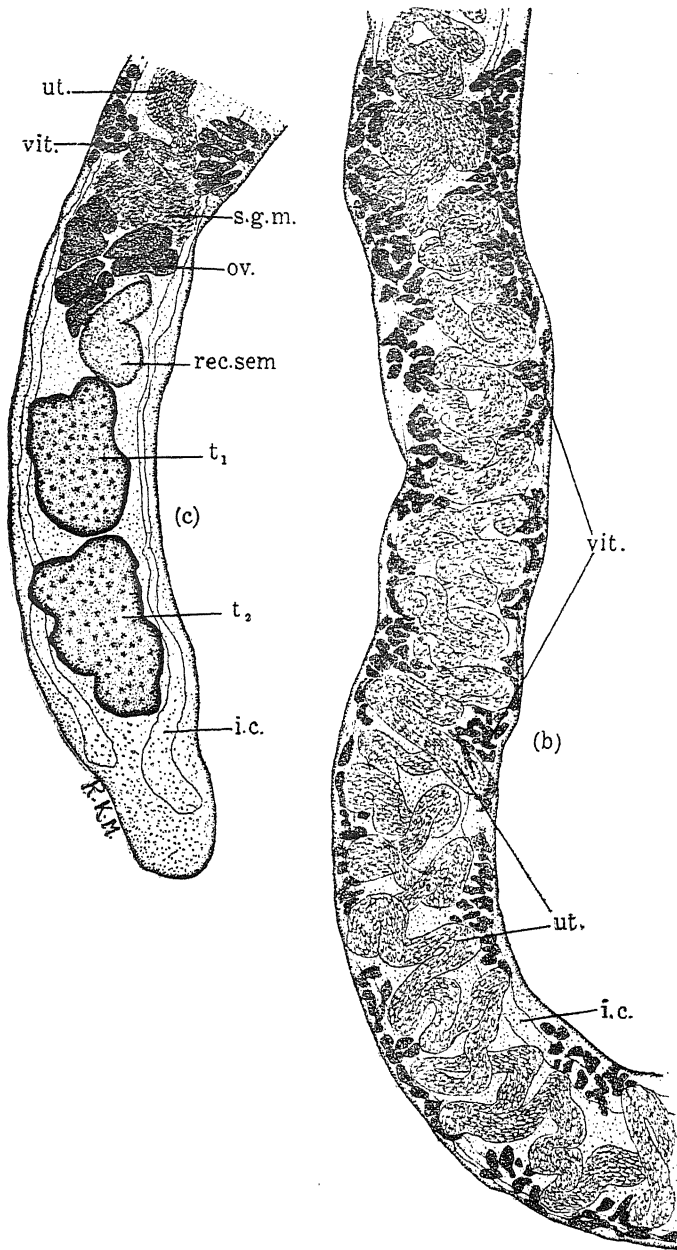
two or three parts and not complete ones. The body is very long, slender and flattened dorso-ventrally, measuring 8.7–16.5 mm. in length and 0.42–0.95 mm. in maximum breadth which lies either in the region of the anterior testis or a little in front of it. The anterior part of the body upto the ventral sucker in smaller specimens and a little in front of the latter in bigger specimens is marked off from the rest of the body by a constriction. This anterior part tapers towards the pointed anterior end. The breadth in the region of the constriction measures 0.33–0.58 mm., behind which it gradually increases till in the region of the vitellaria it runs uniformly 0.39–0.9 mm. and behind the testes narrows into a rounded posterior end. The bodywall is smooth and entirely devoid of spines and also such muscle bands as Yamaguti observed in his species *O. anatis*.

The rounded oral sucker of 0.132–0.165 mm. diameter lies at the pointed anterior end. The prepharynx is absent. The pharynx has 0.145–0.165 mm. length and 0.11–0.135 mm. maximum breadth. The oesophagus is longer than the combined lengths of oral sucker and pharynx, measuring 0.225–0.375 mm. in length and 0.05–0.15 mm. in maximum breadth which lies just before the intestinal bifurcation. The wide intestinal caeca pursue nearly a straight course from their origin lying parallel to the body length and terminate in front of the hinder end, a little behind the posterior testis. The caeca are not equal in length, the right caecum ending in front of the left one. The outline of intestinal walls shows slight irregularities. The acetabulum nearly rounded in shape and equal in size to the oral sucker lies just behind the anterior one-sixth of the body length measuring 0.14–0.18 mm. in diameter.

The testes and ovary with the receptaculum seminis and shell gland mass lie in the posterior one-fourth part of the body length. The unequal testes massive and irregular in outline without any definite shape or form, slightly shifted to the right side lie close behind one another with a small space in between them and sometimes slightly overlapped by the right intestinal caecum. The anterior testis is smaller, 0.56–0.9 mm. in length and 0.36–0.525 mm. in maximum breadth and the posterior 0.57–0.98 mm. in length and 0.36–0.57 mm. in maximum breadth. The number of lobes in each testis varies in different specimens. The long obliquely or transversely coiled and dorsally situated vesicula seminalis of 0.181 mm. diameter which is filled with sperms begins at about the end of the anterior one fourth body length. Its coils are intercæcal and lie intermingled with the uterine coils of that region behind the acetabulum, except the last coil which after passing in front of the latter turns backwards to open into the genital atrium, situated closely in front of the acetabulum. Deeply staining cells are present overlapping and surrounding the acetabulum.

The ovary is tripartite and each part is deeply multi-lobed giving the entire ovary in some specimens a follicular appearance, lies median in the intercæcal space,

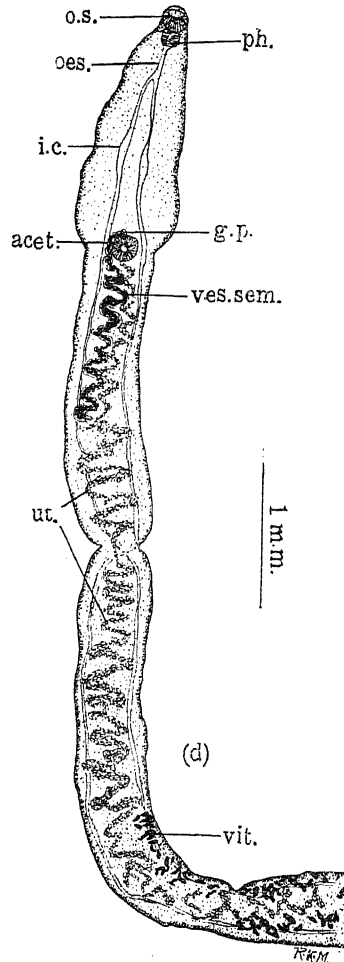
0.13–0.21 mm. in front of the anterior testis, and measures 0.31–0.675 mm. in length and 0.25–0.6 mm. in maximum breadth. The large shell gland mass of an



(b) middle portion showing the extent of vitellaria. (c) posterior one-fourth portion.

(Lettering as in Fig. 1)

irregular form fills the entire intercæcal space closely in front of the ovary, and sometimes overlapping the cæca. The receptaculum seminis is a large elongated, somewhat pear-shaped sac lying intercæcally pressed between the ovary and the anterior testis close inside the left intestinal cæcum, measuring 0.285–0.6 mm. in length and 0.165–0.33 mm. in maximum breadth. The short and narrow Laurer's



(d) anterior portion of a smaller specimen.

(Lettering as in Fig. 1)

canal appears to arise from the centre of the ovary, where it joins the narrow part of the receptaculum seminis a little before the latter opens into the ootype surrounded by the shell gland mass and runs a little backwards to open to the exterior. From the anterior side of the shell gland mass opposite to that at which the oviduct joins

it there arises proximal part of the uterus in two or three irregular coils. The main uterine coils are not opisthorchid in their disposition throughout their entire course. They are, however, broad and irregular upto the commencement of the vitellaria, in front of which they display a more or less spiral arrangement. The last coil runs straight to the left side of the body dorsally to the acetabulum and opens into the genital atrium close to the male opening. The uterus is filled with ova and occupies throughout its length an intercaecal position between the shell gland mass and the acetabulum, sometimes extending on or outside the intestinal caeca. The ova measure 0.0231–0.0264 mm. in length and 0.0125–0.0132 mm. in maximum breadth.

The vitellaria lie laterally, near the bodywall outside and overlapping the intestinal caeca, extending from the commencement of the ovary to about half the distance between it and the acetabulum. Each vitellarium is composed of eight groups of irregularly shaped follicles. The vitelline ducts run longitudinally parallel to the long axis of the body each connecting the vitelline groups of its side. At the posterior margin of the vitellaria these ducts run obliquely backwards and inwards to unite just in front of the ovary to form the dorsally situated yolk reservoir.

The excretory bladder and its cornua could not be seen in the entire mounts.

Remarks:—This species differs from all the known species of the genus in having its anterior part of the body separated into a cephalic region. It resembles *O. longissimus* (v. Linstow 1883) as defined by Erhardt (1935) in the size of the body, absence of the prepharynx, shape of the testes, multi-lobed condition of the ovary and in the extent of the vitellaria. But it differs from it in both the suckers being equal in size (in *O. longissimus* the oral sucker is either smaller or larger than the ventral sucker), and in having the genitalia in the hinder fourth of the body instead of further behind, i.e., in the fifth to the seventh part as in *O. longissimus*.

Opisthorchis indicus n. sp. (Fig. 3.)

Only two mature specimens were obtained from the gall bladder of a Goshawk *Austur gentilis gentilis* caught at Allahabad. The body is elongated, slender and delicate, with a slight constriction just behind the acetabular zone, measuring 7 mm. in length, 1.215 mm. in maximum breadth which lies throughout the length behind the constriction, 1.14 mm. in the region of the genital opening, and 1.65 mm. at the constriction. The bodywall is devoid of spines.

The oval, transversely elongated oral sucker is situated subterminally at the bluntly pointed anterior end, measuring 0.24–0.255 mm. in length and 0.21 mm. in maximum breadth. The prepharynx is absent. The muscular pharynx is distinctly larger than half the size of the oral sucker measuring 0.152–0.165 mm. in length and 0.115 mm. in maximum breadth. The oesophagus is well developed, measuring 0.3–0.42 mm. in length and 0.112 mm. in maximum breadth. The intestinal bifurcation lies 0.6–0.78 mm. away from the anterior end. The

intestinal cæca soon after their origin run obliquely pursuing a straight course behind the acetabulum parallel to the body length as far as the anterior testis behind which they slightly bend inwards terminating near each other a little in front of the

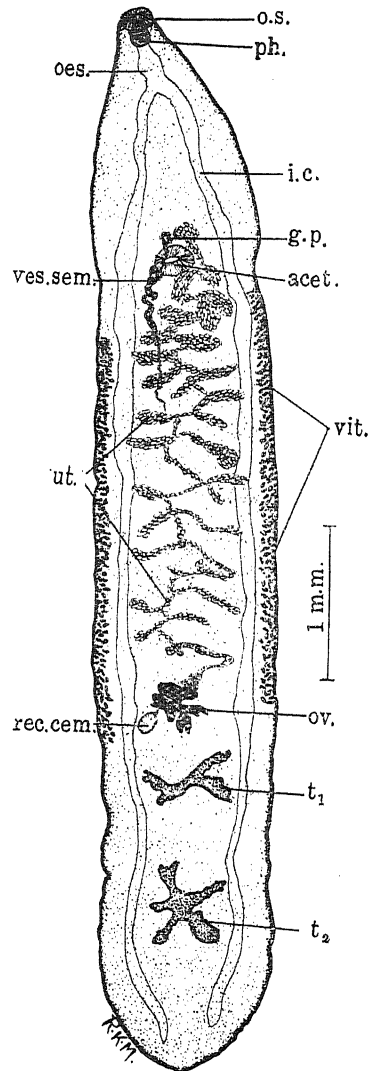


Fig 3 —Ventral view of *Opisthorehis indicus* n. sp.

(Lettering as in Fig. 1)

hinder end. The acetabulum which is rounded in one specimen measuring 0.27 mm. in diameter and oval in the other measuring 0.21–0.285 mm. in size lies far behind the intestinal bifurcation at the end of the anterior one-fourth body length.

The excretory system could not be studied in the living condition and it is not visible in entire mounts.

The genital opening lies as usual in the genus closely in front of the acetabulum, at 0.1 mm distance from it and 1.5 mm. away from the anterior end. The testes lie tandem in the posterior one-third of the body length between the intestinal cæca, anterior testis at 0.42 mm. in front of the posterior testis and the posterior at 0.6—0.75 mm. in front of the hinder end of the body. They are not massive but dendritic or deeply lobed. The anterior testis consists of four and the posterior five lobes which again have got nodules on them. The small thin walled vesicula seminalis 0.066 mm. in maximum width, lies freely in the parenchyma to the right of the median line. It commences as a fine straight tube at about 1.08 mm. behind the acetabulum, near which it becomes thicker and coiled. After passing dorsally to the acetabulum it opens into the genital atrium. The cirrus sac and prostate glands as usual in the family are absent.

The ovary consisting of three parts of which each is further lobed lies slightly to the right side of the median line at 0.24—0.3 mm. in front of the anterior testis. Each of the three lobes of the ovary is divided first into two and then four or more parts. The small, indistinct shell gland mass composed of a few shell gland cells lies just anterior to the ovary. The Laurer's canal was not seen in this species. The small oval receptaculum seminis, 0.165 mm. in length and 0.1 mm. in maximum breadth, lies to the right side of the ovary very close to the right intestinal cæcum. It is connected with the oviduct by a small narrow duct. The uterus arises from the anterior lobe of the ovary and runs forwards in the thin walled, obliquely arranged coils which lie far from each other within the intercæcal space sometimes overlapping the cæca but never extending outside them. In the acetabular region the coils are confined only to the left side of the median line. The uterus opens after extending a little in front of the acetabulum into the genital atrium. The metraterm is absent. The ova are numerous and oval in shape, measuring 0.0264 mm. in length and 0.0132 mm. in maximum breadth.

The vitellaria lie laterally near the body wall in the form of bands outside the intestinal cæca from which they are separated by a narrow space. They, though of equal length do not lie at the same level, the left commencing and ending a little ahead of the right one, *i.e.*, commencing from the posterior margin of the acetabulum and ending at the anterior end of the ovary. The right vitellaria ends at the posterior end of the ovary. They are composed of numerous, minute irregular vitelline follicles which lie close to one another and are not arranged in ascini. The transverse vitelline ducts and yolk reservoir could not be seen.

Remarks :—This species differs from all the other known species of the genus in the shape of the body, shape of the ovary, and the position and structure of vitellaria which are composed of minute follicles lying close to the body wall. It

combines in itself some characters of *O. sinensis* (Cobb. 1875) and *O. obsequens* Nicoll 1914. It resembles the former species in showing the tandem position of the ovary and testes, in the extent of the vitellaria upto the region of the ovary and in the trilobed condition of the ovary, while it resembles the latter in having the acetabulum larger than the oral sucker, oesophagus longer than the combined lengths of the oral sucker and pharynx and in the shape of the testes. It, however, differs from both in the shape of the body which has a constriction behind the acetabular region, on account of the characteristic multilobed ovary, in the extremely small size of the receptaculum seminis and the vitellaria being situated at different levels.

Erhardt 1935 considers *O. sinensis* and *O. obsequens* to be so closely related as to be included in the same "Artenkreis" (after Rensch). The new species which also comes under the same group should be designated as *O. sinensis indicus* n. sp.

Opisthorchis pelecani n. sp. (Fig. 4.)

Three specimens of this form were obtained from the gall bladder of a Rosy pelecans *Pelecanus onocrotalus onocrotalus* which died in the Zoology Department of the Allahabad University in May 1940 after remaining in captivity for about twenty days. In living condition the worms were pink in colour, long, slender and dorso-ventrally flattened with bluntly pointed anterior end and rounded posterior end. The body in entire mounts measures 5-6.5 mm. in length and 0.705-0.8 mm. in maximum breadth which occurs at about the middle of body length. The breadth of the body gradually increases from the anterior end upto the commencement of the vitellaria behind which it remains more or less uniform measuring 0.4-0.45 mm. in the region of the intestinal bifurcation and 0.5-0.66 mm. in the acetabular region. The entire integument is beset with minute spines which give the body surface a serrated appearance specially in the posterior one third.

The oval oral sucker situated at the bluntly pointed anterior end measures 0.132-0.135 × 0.1-0.105 mm. in size. The prepharynx is absent. The rounded muscular pharynx measures 0.086-0.09 mm. in diameter. The oesophagus measures 0.23-0.29 mm. in length and 0.05-0.064 mm. in maximum breadth. The intestinal caeca soon after the bifurcation run posteriorly nearly parallel to the body length, terminating a little in front of the hinder end where they come close to each other. The ventral sucker is nearly rounded and situated just behind the anterior third of body length, measuring 0.18-0.192 mm. in size in all the three specimens obtained.

The genitalia lie in the hinder fourth part of the body. The lobed testes, massive, similar in shape, each composed of five lobes, lie tandem between the intestinal caeca and slightly overlapping them. The anterior testis of 0.39-0.465 × 0.225-0.255 mm. size lies 0.08-0.1 mm. in front of the posterior testis, which measures 0.39-0.465 × 0.24-0.255 mm. in size and is situated 0.33 mm. in front of

the hinder end of the body. The long coiled vesicula seminalis begins in front of the anterior end of the vitellaria and runs anteriorly to open into the genital atrium situated medianly close in front of the acetabulum.

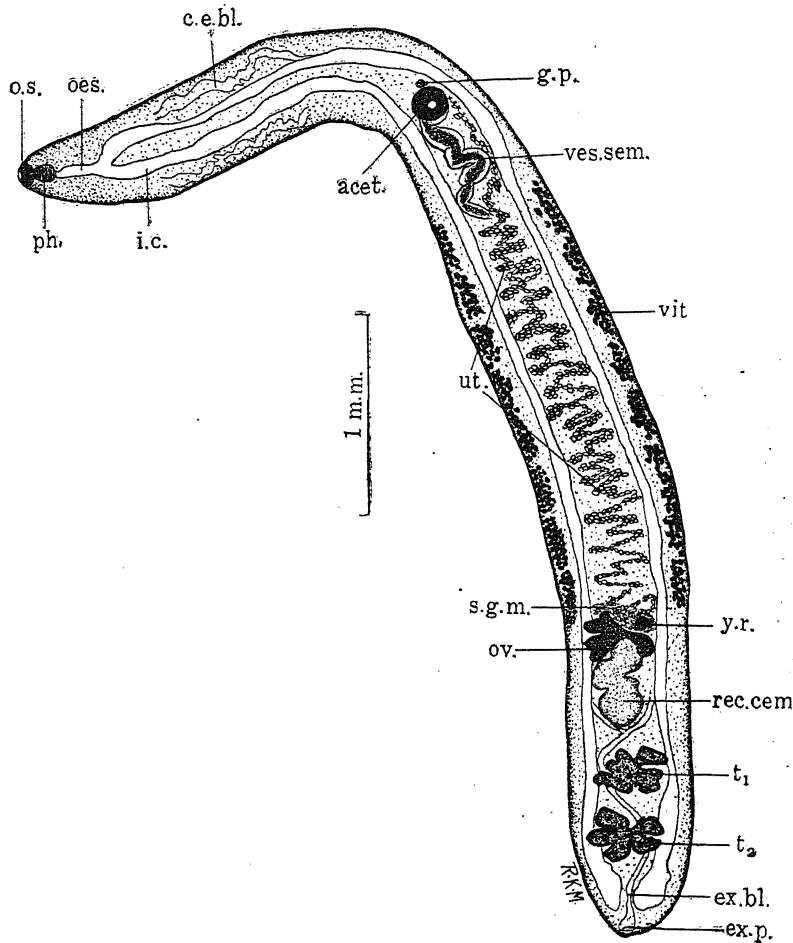


Fig. 4 —Dorsal view of *Opisthorchis pelecani* n. sp.

(Lettering as in Fig. 1)

The trilobed ovary slightly shifted towards the left side in the intercæcal space and situated 0.18–0.405 mm. in front of the anterior testis measures 0.375–0.405 mm. in length and 0.12–0.24 mm. in maximum breadth. The two of the three lobes of the ovary are bifid. The small diffused shell gland mass occupies the intercæcal space just in front of the ovary. The large elongated somewhat S-shaped receptaculum seminis of 0.35 × 0.24 mm. size lies in the intercæcal space just behind the ovary

near the left intestinal caecum. The Laurer's canal opens to the exterior on the right side of the receptaculum seminis. The uterus as it arises from the shell gland mass runs forward in the characteristic strictly intercæcal opisthorchid coils confined between the ovary and the commencement of the vesicula seminalis. Its short terminal part, however, more or less straight runs parallelly to the body length on the right side of the vesicula seminalis and the acetabulum opening in the genital atrium close to the male opening. The ova of $0.023-0.0264 \times 0.0165$ mm. size are numerous and oval in shape.

The vitellaria composed of eight groups of follicles of irregular shape lie laterally one on each side, in the form of bands between the body wall and the intestinal caeca, extending from the commencement of the vesicula seminalis to the anterior margin of the ovary. The transverse vitelline ducts arise from the hinder ends of the vitellaria, run inwards and unite near the right caecum to form the dorsally situated yolk reservoir which lies just in front of the ovary.

Remarks :—So far only one species from the avian host with spines on the body surface *O. speciosus* Stiles and Hassals 1896 is known, whereas the other species with cuticular spines are from mammalian and piscine hosts. *O. pelecani* n. sp. differs remarkably from *O. speciosus* which belongs to the subgenus *Amphimerus* (Barker 1911) Erhardt, 1935 and is separated from the subgenus *Opisthorchis* R. Blanchard 1895 on account of the extent of the vitellaria, which do not extend even behind the ovary (in *Amphimerus* vitellaria extend posteriorly upto hinder testis or even further behind).

O. pelecani n. sp. which resembles *O. viverrini* and *O. noverca* of mammalian hosts and *O. pedicellata* and *O. piscicola* of piscine hosts in having spines on the body surface and in the extension of the vitellaria upto the ovary, differs from the first three species in having the oral sucker smaller than the ventral sucker (in *viverrini*, *noverca*, and *pedicellata* oral sucker is equal to or larger than the ventral sucker) in which it resembles *piscicola*. The last species and *O. pelecani* n. sp. also differ from *viverrini*, *noverca* and *pedicellata* in the shape and size of the body, testes, ovary and receptaculum seminis and in measurements of other organs. The new species also comes closer to *O. piscicola* in the median position and lobed condition of the testes and ovary. But it differs from it in having extremely small spines, in the greater length of the body, deeply lobed condition of the testes characteristic shape of the ovary and larger size of the ova.

References are given at the end of Part II.

A CONTRIBUTION TO THE STUDY OF THE GENUS *OPISTHORCHIS*
R. BLANCHARD 1895. PART II. DESCRIPTION OF SUBSPECIES
AND DISCUSSION ON THE SYNONYMY OF *GOMTIA*
THAPAR, 1930 AND *OPISTHORCHIS* R. BLANCHARD
1895, WITH KEY TO THE SPECIES OF THE
GENUS *OPISTHORCHIS*.

BY RAM KRISHNA MEHRA.

ZOOLOGY DEPARTMENT, UNIVERSITY OF ALLAHABAD.

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SUMMARY

One already known sub-species and a new sub-species of the genus *Opisthorchis* R. Blanchard 1895 are described. The synonymy of the genus *Gomtia* Thapar, 1930 with *Opisthorchis* is discussed and a note on the former is appended. The key to the species of the genus *Opisthorchis* as given by Erhardt is amended.

Opisthorchis tenuicollis geminus (Looss, 1896) Erhardt 1935.

(Figs. 1 and 2)

Large number of specimens of this sub-species were obtained from the gall bladder and liver of kites, *Milvus migrans govinia* and vultures, *Sarcogyps calvus* dissected at Allahabad during the last two years. The sub-species was first described by Looss from Egypt and again in 1910. The specimens collected by me are either smaller or larger in size than those of Looss and also differ in some minor points. The following note is, therefore, given to add to the description of these distomes already known. The body measures 6–11.5 mm. in length and 1.6–2.355 mm. in maximum breadth at about middle of the body length. The oval oral sucker is subventrally situated at the pointed anterior end and measures $0.18 \times 0.24 - 0.285$ mm. in size. The muscular pharynx has $0.12 - 0.165 \times 0.15 - 0.21$ mm. size. The thin-walled oesophagus of 0.53–0.6 mm. length bifurcates at 0.7–1 mm. distance in front of the acetabulum into undulating cæca which terminate with their blind ends bending inwards close to one another separated only by the excretory pore.

The excretory system corresponds to that described by Looss. The S-shaped excretory bladder passes between the two testes and divides just behind the receptaculum seminis into two short cornua. The testes slightly or deeply lobed, lie obliquely in the posterior one-sixth part of body-length. The testes are unequal in

smaller specimens. The anterior testis is smaller and measures $0.33-0.48 \times 0.59-0.7$ mm. in size and the posterior $0.405-0.525 \times 0.525-0.78$ mm. in size. In the larger specimens the testes are equal, measuring 0.78×0.96 mm. in size. The number

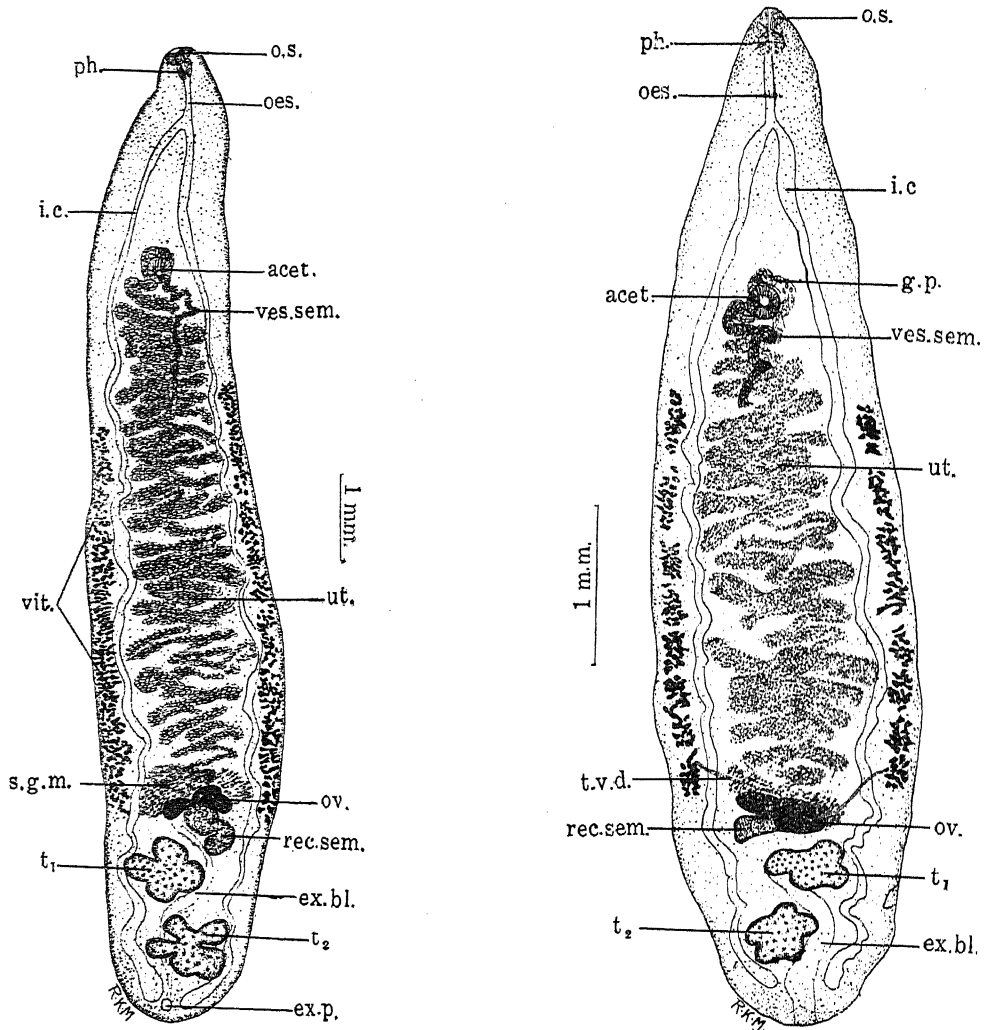


Fig. 1.—Dorsal view of *Opisthorchis tenuicollis geminus* (Looss, 1896)

Fig. 2.—Ventral view of *Opisthorchis tenuicollis geminus* (Looss, 1896)

acet., acetabulum; *ex. bl.*, excretory bladder; *ex.p.*, excretory pore; *g.p.*, genital pore; *i.c.*, intestinal caecum; *l.c.*, Laurer's canal; *oes.*, oesophagus; *o.s.*, oral sucker; *ov.*, ovary; *ph.*, pharynx; *p. ph.*, prepharynx; *rec. sem.*, receptaculum seminis; *s.g.m.*, shell gland mass; *t1*, anterior testis; *t2*, posterior testis; *t.v.d.*, transverse vitelline duct; *ut.*, uterus; *vit.*, vitellaria; *ves. sem.*, vesicula seminalis; *y.r.*, yolk reservoir.

of lobes in each testis is constant, *i.e.*, four in the anterior and five in the posterior testis. The slightly coiled tubular vesicula seminalis commences at the anterior level of the vitellaria and runs forwards to the right side to open into the genital atrium which lies closely in front of the acetabulum.

The trilobed ovary, $0.78-0.84 \times 0.21-0.24$ mm. in size, lies median or slightly to the right side a little in front of the anterior testis. The three lobes of the ovary are nearly equal in size. The large shell gland mass occupies the intercæcal space just in front of the ovary. The long and narrow Laurer's canal opens dorsally in the region of the receptaculum seminis. The uterus compactly filled with ova is transversely coiled and opens in the genital atrium near the male opening. The ova measure 0.0264×0.0132 mm. in size.

The vitellaria are extracæcal and composed of eight follicle groups which may or may not be quite separate from one another. They commence at some distance behind the acetabulum. The transverse vitelline ducts arise from the hinder end of the vitellaria and join dorsally to form the yolk reservoir just in front of the left lobe of the ovary.

Of the two species described by Lal (1939) *O. giddhis* and *O. cheelis* from *Sarcogyps calvus* and *Milvus migrans* respectively, *i.e.*, the same hosts from which my specimens were also obtained, *O. cheelis* Lal is identical without any doubt and, therefore, synonymous to *O. t. geminus* because the specific characters mentioned are such as have no systematic value according to the "The individual variations" in the morphology of the genus *Opisthorchis* by Erhardt (1935). The two characters, *i.e.*, the presence of an extremely small prepharynx and spines in the anterior part of the body in *cheelis* have not been noticed by me in the specimens described above.

The prepharynx is not shown in the diagram given by Lal nor, does he mention anything about its size. Slight shifting of the pharynx due to pressure in flattening may take place in such a way as to separate it from the oral sucker thus give an idea about the presence of an extremely small prepharynx. My observations based on a large number of living specimens as well as toto mounts lead me to conclude that it is entirely absent. As has been pointed out by Erhardt (1935) ordinarily the cutaneous spines in the species in which they do not occur disappear after the twentieth day of infection as in *O. t. tenuicollis* (Rud. 1819). It is therefore likely that the spines in *O. cheelis* are present only in relatively early condition of infection and in fully mature specimens they disappear.

The validity of *O. giddhis* Lal is also doubtful, but as its author definitely mentions the presence of a thick-walled oesophagus and a prepharynx of 0.05 mm. length the species is retained till more material is obtained from *Sarcogyps calvus*.

Opisthorchis pedicellata minuta n. subsp.

(Fig 3)

About twenty specimens of this form were obtained from the gall bladder of the fish *Mystus seenghala* Sykes and *Vallago attu* Bleeker called in vernacular *Tengra* and *Parhna* respectively which were obtained from Ganges and Jamuna at

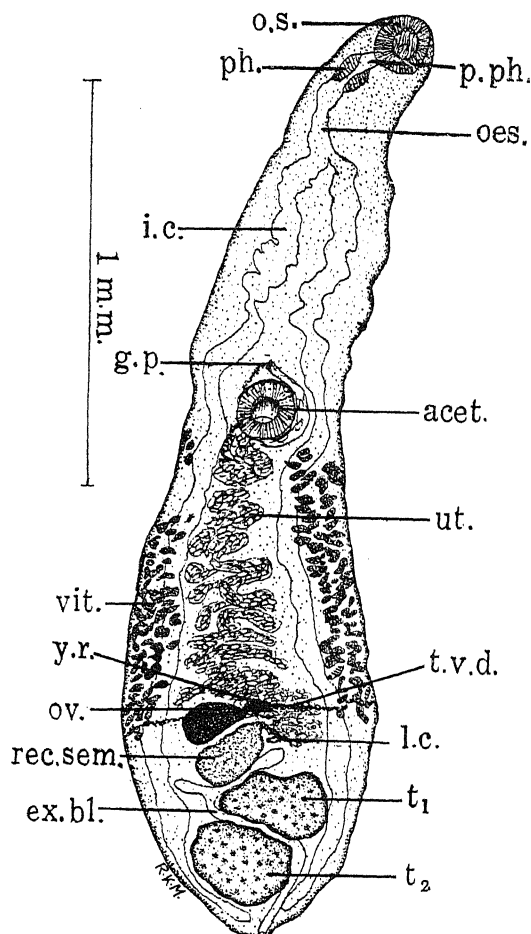


Fig. 3.—Ventral view of *Opisthorchis pedicellata minuta* n. subsp.

(Lettering as in Fig. 1)

Allahabad. Verma in 1927 described *Opisthorchis pedicellata* from *Rita rita* (= *Rita buehanani* Day) and *Bagarius yarrellii* (= *Finelodus bagarius* Ham. Buch.) but he did not find these parasites in any other species of the local fish, though he examined most of them. All the specimens obtained from the above-mentioned

hosts are fully mature but they are much smaller in size than those obtained by Verma and show some other differences which clearly entitle this form to the rank of a new sub-species of *Opisthorchis pedicellata* Verma.

The body measures, 2.4–5 mm. in length and 0.56–0.84 mm. in maximum breadth which lies either in the region of the ovary or a little in front of it. The breadth in the region of the ventral sucker is 0.33–0.56 mm. All these measurements of length and breadth vary according to the contraction and expansion of the specimens. The surface of the body is covered with very minute spines upto the acetabular zone behind which they become sparse. The spines 0.0132 mm. in length, are so small that they are not easily visible under the low power of the microscope.

The rounded oral sucker, 0.106–0.181 mm. in diameter, is situated subventrally at the bluntly pointed anterior end. The ventral sucker is also rounded, measuring 0.119–0.181 mm. in diameter and lies 0.975–1.95 mm. behind the anterior end of the body, *i.e.*, often at about the end of anterior one-third body length. In some specimens, however, it is also situated in the middle of the body. The two suckers are hence equal in size and similar in shape even in quite mature specimens. In *O. pedicellata* according to Verma the suckers are unequal in mature specimens although for immature specimens he says "But in immature flukes the two suckers are either of the same size (*vide* Table IV, last example), or the condition is reversed, the ventral sucker being decidedly smaller than the oral."

The oral sucker opens into a very small thin-walled prepharynx which leads into the elongated muscular pharynx of 0.073–0.119 mm. length and 0.056–0.083 mm. maximum breadth. The oesophagus whose length varies according to the contraction of the anterior part of the body does not measure less than twice the length of the pharynx. The caeca having greater breadth than the oesophagus are crenated upto the acetabulum behind which they pursue nearly a straight course parallel to the body length, terminating at the same level near the hinder end of the body.

The testes and the ovary lie in nearly all the specimens in the posterior one-fifth part of the body. The smaller anterior testis, 0.165–0.33 mm. in length and 0.24–0.375 mm. in breadth, is nearly triangular in shape and occupies the same position as described by Verma. The posterior testis of nearly rounded shape lies 0.075–0.3 mm. in front of the hinder end and measures 0.2–0.405 mm. in length and 0.21–0.39 mm. in maximum breadth. Both the testes have got irregular margins but they are not distinctly lobed as in *Opisthorchis pedicellata* Verma. The two testes in majority of specimens are very close to each other while in a few they are clearly separated from one another by the thickness of excretory bladder as Verma noticed in the young specimens of his form. The vesicula seminalis is a thin walled coiled tube full of sperms lying mostly in between the uterine coils behind the acetabulum. It passes to the right side of the acetabulum and a little

beyond it and then turns inwards and slightly backwards to open into the genital atrium. The prostate gland cells are absent.

The ovary in almost all the specimens is pear-shaped, measuring 0.15–0.3 mm. in length and 0.105–0.24 mm. in maximum breadth. It lies transversely to the right side between the caeca 0.48–0.82 mm. in front of the hinder end. The small diffused shell gland mass occupies nearly the same position on the left side of the body as the ovary does on the right side. The large and pear-shaped receptaculum seminis of 0.15–0.3 mm. length and 0.075–0.18 mm. maximum breadth, *i.e.*, nearly equal in size to the ovary or sometimes even larger lies immediately behind the latter and towards the right side of the body. The Laurer's canal, 0.026 mm. in maximum breadth, opens to the exterior near the pointed narrow end of the receptaculum seminis. The uterus arises from the shell gland mass and runs forward in the characteristic opisthorechid uterine coils whose number varies much in different specimens. The number of uterine coils may be ten or twelve only or large when they are closely packed together filling up the entire intercaecal space. Terminally the uterus runs along the left side of the acetabulum and then after turning a little towards the median line it opens into the genital atrium near the male opening, closely in front of the acetabulum. The uterus is filled up with eggs of oval shape, which measure 0.0297 mm. in length and 0.0132 mm. in maximum breadth. The colour of the mature ova is yellow and not reddish brown as in the type species.

The vitellaria lie laterally in the form of bands outside and overlapping the intestinal caeca and are composed of a large number of small follicles of irregular shape. They commence just behind the acetabulum and terminate at the posterior margin of the ovary. The transverse vitelline ducts arise a little in front of their posterior end and run inwards and forwards to open into a small vitelline reservoir which lies dorsally near the narrow anterior end of the ovary.

Remarks.:—This subspecies resembles the type species *O. pedicellata* Verma in the general topography. But it differs from it mainly in the size of the body which is very small, the internal organs being correspondingly reduced in size, in having the testes with crenated margins and not distinctly lobed and in the shape of the receptaculum seminis which is pear-shaped and not oval. The size of the two suckers in the mature specimens of the new subspecies is nearly equal whereas in the type species it is not so, the ventral sucker being much larger than the oral sucker.

These differences though not sufficient for the creation of a new species justify the creation of a subspecies within "Rassenkreiss" *pedicellata*. The type species *O. pedicellata* recognised as *O. pedicellata pedicellata* (Verma 1927) is distinguished from *O. pedicellata minuta* nov. subsp. on account of the size of the body and ratio in the size of the suckers.

ON THE SYNONYMY OF *GOMTIA* THAPAR, 1930 AND *OPISTHORCHIS*

R. BLANCHARD, 1895.

Thapar in 1930 created a new genus *Gomtia* for the specimens obtained by him from the Siluroid fishes in the river Gomti at Lucknow. This genus according to him occupies an intermediate position between *Opisthorchis* and *Notaulus* Skrjabin, 1913. Morgan in 1927 had dropped the genus *Notaulus* and assigned the species *N. asiaticus* to the genus *Opisthorchis*. Though Erhardt (1935) remarked that the genus *Opisthorchis* as defined by him also contained *Gomtia* but he put it as a separate genus in the subfamily Opisthorchinae along with *Opisthorchis*. He reduced *Notaulus asiaticus* to the rank of a subspecies under *Opisthorchis logissimus* (v. Linstow, 1883) Erhardt 1935. Tubangui and Masilungan (1935), while they do not say anything about Morgan's view regarding *Notaulus* and on the validity of the genus *Gomtia*, compared their new species *Opisthorchis ophidiarum* with *Gomtia piscicola* saying that in the presence of long prepharynx and cuticular spines and in the shape and position of its ovary, testes and excretory bladder it is clearly related to the latter form. They obviously considered *Gomtia* to be identical and synonymous with *Opisthorchis*, though they did not mention it. After studying closely the two specimens of *G. piscicola* collected from the intestine of a fish *Bagarius yarrelli* I am convinced of the identity of the two genera.

The genus *Gomtia* was based on the following characters:—

- (i) Body covered with fine spines.
- (ii) Presence of a long prepharynx.
- (iii) Excretory bladder straight and not S-shaped as in the genus *Opisthorchis*.
- (iv) Testes, nearly rounded and situated at the extreme posterior end with ovary a little in front of them, all in a linear series.
- (v) Position of vitellaria between $\frac{2}{3}$ and $\frac{3}{4}$ part of the body length.

All these characters are met with in one or the other species of the genus *Opisthorchis*. In many species the entire body is covered with spines and this character has been utilised by Verma (1927) and Erhardt (1935) for the separation of species in their key of the genus *Opisthorchis*. The long prepharynx though not known to be present in any species of *Opisthorchis* before Thapar described *Gomtia*, has even greater length in *O. ophidiarum*. Tubangui and Masilungan obviously did not consider this character of generic importance when found that their species agreed with *Opisthorchis* in many characters and I also agree with the latter authors on this point.

Morgan remarks that the excretory bladder when pressed dorsally by the testes does not remain sigmoid but becomes straight. In *O. felineus*, however, the sigmoid excretory bladder according to him is overlapped by the branches of the testes. In my opinion, the large massive testes filling almost the entire breadth at hinder end of body do not allow any space for the excretory bladder to take a

sigmoid form. It is, therefore, in such species where this condition is met with that the excretory bladder is straight and dorsal in position as in *O. ophidiarum*, *O. l. asiaticus* (Skrjabin, 1913) Erhardt 1935 and *Gomtia piscicola*. In a few cases the excretory system is not even properly seen as in *O. anatis* Yamaguti 1933. As Morgan was justified in transferring *Notaulus asiaticus* which has a straight excretory bladder to the genus *Opisthorchis* I also attach no generic importance to this character in retaining the genus *Gomtia*.

The testes are rounded in many species of the genus, the rounded shape of testes, therefore, is at the most of specific importance. The linear arrangement of the gonads due to the body being narrow at the hinder end is known in several species such as *O. ophidiarum*, *O. anatis*, *O. pseudofelineus* Ward, 1901 and *O. ahingii* n. sp. Also the position of vitellaria in *Gomtia* is quite similar to that in several species though their extent varies from the hinder end of testes to the acetabulum. So their extent between $\frac{2}{3}$ and $\frac{1}{3}$ of the bodylength should not be considered a character of generic rank.

From the foregoing it, therefore, follows that the genus *Gomtia* should not be recognised as a separate genus and *G. piscicola* should come under the genus *Opisthorchis*. As *Opisthorchis piscicola* is already occupied, *Gomtia piscicola* Thapar is therefore replaced by *Opisthorchis gomtii* nom. nov. (Thapar, 1930) a note on which follows.

Opisthorchis gomtii nom. nov. (Syn. *Gomtia piscicola* Thapar, 1930).

(Fig. 4).

Two specimens of this species were obtained from the intestine of a fish *Bagarius yarrellii* Sykes. Though they agree with most of the important characters of the above-named species they show some differences in the details of anatomy and size of organs.

The size is large than that mentioned by Thapar measuring 2.65–3.8 mm. in length and 0.735 mm. in maximum breadth which lies a little behind the acetabulum. The spines are not present over the whole body as stated by Thapar. They are present in large numbers in anterior part of body upto the hinder end of the acetabulum behind which they become sparse, disappearing altogether in the posterior region behind the ovary. They measure 16–23 μ in length.

The rounded subterminal oral sucker is not larger than the ventral sucker, as mentioned by the previous author but in my specimens it is just the reverse, i.e., the oral sucker measures 0.086–0.1056 mm. and ventral sucker 0.116–0.14 mm. in diameter. The oral sucker leads into the long prepharynx of 0.089–0.178 mm. length. The muscular pharynx which measures 0.066–0.072 mm. in length and 0.053–0.063 mm. in maximum breadth is smaller in size than in the specimens

described by Thapar. The œsophagus is nearly equal to the prepharynx in length and divides 0.2–0.33 mm. in front of the acetabulum into the intestinal cæca which run nearly parallel to the body length behind the latter, terminating just in front of the posterior testis.

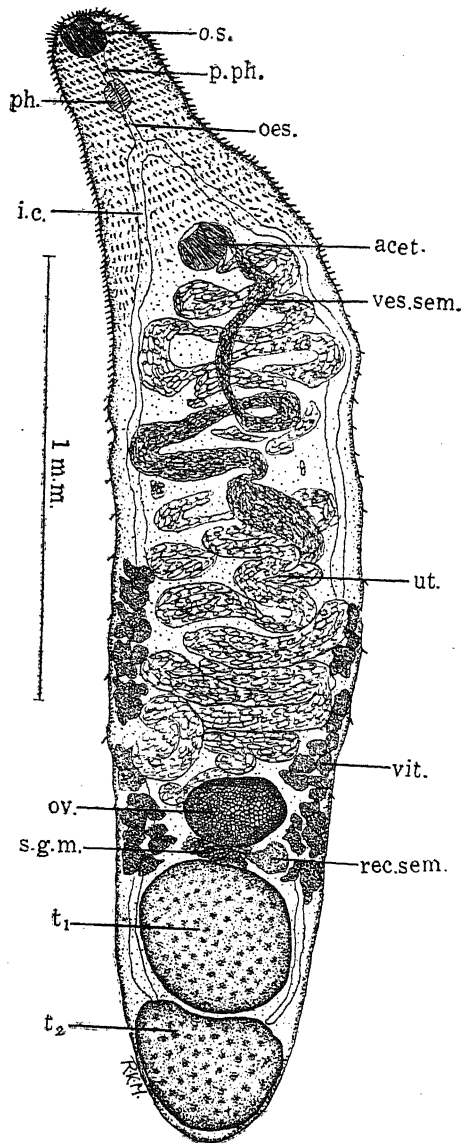


Fig 4.—Ventral view of *Opisthorchis gomtii* nom. nov. (Thapar, 1930).

(Lettering as in Fig. 1)

The excretory system is similar to that described by Thapar.

The testes and ovary are situated in the posterior one-third body length. The large massive testes with entire margins differ in shape and lie tandem in the hindermost part of the body. The anterior testis is larger, rounded in shape and measures 0.33–0.405 mm. in diameter. The posterior testis is somewhat triangular and situated immediately behind the anterior testis, measuring 0.24–0.33 × 0.27–0.33 mm. in size. The long vesicula seminalis filled with sperms commences just in front of the vitellaria, makes a few transverse coils between caeca and then runs straight to open into the shallow genital atrium which lies closely in front of the acetabulum.

The ovary, 0.18–0.225 mm. in length and 0.15–0.165 mm. in maximum breadth, is simple, entire, oval in shape and situated slightly to the right side close in front of the anterior testis from which it is separated by the narrow space occupied by the receptaculum seminis. It is larger in size than in the specimens described by Thapar and also differs slightly in shape. The receptaculum seminis measures 0.1155–0.185 × 0.0825–0.0891 mm. in size. The shell gland mass occupies the narrow space between the ovary and anterior testis. The uterus as it arises from the shell gland mass runs in front of the ovary in transversely arranged and closely packed coils upto the anterior limit of the vitellaria, but further in front the coils are separated and loosely arranged. The uterine coils occupy almost the entire intercæcal space, sometimes overlapping the caeca but never extending outside them. The numerous ova fill the entire uterus, are operculate and measure 0.0264 × 0.0132 mm. in size.

The vitellaria are situated laterally outside the intestinal caeca in the posterior half of the body between the anterior testis and middle of body length. Each gland is composed of irregular follicles which never exceed more than thirty in number. The transverse vitelline ducts lie obliquely behind the ovary and unite just above the shell gland mass to form the yolk reservoir.

KEY TO THE SPECIES OF THE GENUS *OPISTHORCHIS* GIVEN BY ERHARDT IS
AMENDED AS FOLLOWS:

- | | |
|---|----------------------------------|
| 1. Vitellaria do not extend upto hinder testis | Subgenus <i>Opisthorchis</i> . 2 |
| Vitellaria extend at least on one side of body to hinder testis or further behind | subgenus <i>Amphimerus</i> . 20 |
| 2. Body with spines | 3 |
| Body without spines or unarmed | 10 |
| 3. Prepharynx present | 4 |
| Prepharynx absent | 8 |
| 4. Oesophagus thickwalled | <i>giddhis</i> Lal. |
| Oesophagus thinwalled | 5 |

5. Ventral sucker distinctly smaller than oral sucker *ophidiarum* Tubangui
and Masilungan.
Ventral sucker equal to or larger than oral sucker 6
6. Vitellaria commence just behind ventral sucker *pedicellata* Verma . . . 7
Vitellaria commence far behind ventral sucker *gontii* nom. nov. (Thapar)
7. Length at least 7 mm., ventral sucker much larger than oral sucker . . .
p. pedicellata
Length at most 5 mm., ventral sucker nearly equal to oral sucker . . .
p. minuta n. subsp.
8. Oral sucker larger than ventral sucker . . . *noverca* Braun
Oral sucker as large as ventral sucker . . . *viverrini* (Poirier)
Oral sucker smaller than ventral sucker 9
9. Testes and ovary slightly lobed, parasitic in fishes *piscicola* Odhner
Testes and ovary deeply lobed, parasitic in birds *pelecani* n. sp.
10. Vitellaria extend anteriorly nearly upto ventral sucker 11
Vitellaria extend anteriorly upto a point which lies at least at $\frac{1}{3}$ or at most
at $\frac{1}{2}$ distance between ovary and acetabulum from the latter . . . 16
11. Testes more or less lobed 12
Testes more or less branched 14
12. Ovary trilobed, each lobe simple . . . *tenuicollis* (Rud.) . . . 13
Ovary trilobed, each lobe again bifid . . . *allahabadii* n. sp.
13. In marine mammals *t. tenuicollis*
In land mammals *t. felineus* (Riv.)
In birds *t. geminus* (Looss)
14. Ovary trilobed 15
Ovary multilobed *indicus* n. sp.
15. Length at most 6 mm., in mammals . . . *sinensis* (Cobb.)
Length at least 5 mm., in birds . . . *obsequens* Nicoll
16. Prepharynx absent 17
Prepharynx present *entzi* (v. Ratz.)
17. Anterior part of body separated by constriction and suckers nearly
equal *ahingii* n. sp.
Anterior part of body not separated, suckers
unequal *longissimus* (v. Linst.) . . . 18
18. Anterior end of vitellaria in hinder body half . *l. dendreticus* Morgan
Anterior end of vitellaria in anterior body half 19
19. Slightly lobed testes between intestinal
caeca *l. longissimus* (v. Linst.)
and *l. simulans* (Looss)
Strongly branched testes overlap intestinal caeca . *l. asiaticus* (Skrjabin).

A PHYSIOLOGICAL STUDY OF *SAPROLEGNIA DELICA* COKER.

By R. K. SAKSENA AND K. S. BHARGAVA.

DEPARTMENT OF BOTANY, UNIVERSITY OF ALLAHABAD.

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SUMMARY

1. *Saprolegnia delica* is capable of unlimited growth in a nutrient medium, containing mineral salts and pure dextrose, which does not contain any significant amount of growth-promoting substances. The results of the experiments show that the organism is capable of synthesizing its own growth-promoting substances from the simple ingredients of the nutrient medium. Such growth-promoting substances manufactured by the organism are also given off by the mycelium into the solution.

2. Addition of vitamin B₁ (Thiamin) to the nutrient medium has no marked effect on the growth of the fungus.

3. The fungus is capable of utilizing sulphur from cystine and not from sulphates. The minimum quantity of cystine required for the growth of the fungus in the nutrient medium is 0.0005 gm. per litre.

4. Of the 15 carbohydrates and 5 Alcohols tested as source of carbon for the fungus, only dextrose, levulose, maltose, dextrin, glycogen, and soluble starch are utilized and are most favourable for the growth and acidification. Arabinose, galactose, mannose, rhamnose, xylose, lactose, sucrose, raffinose, inulin, glycerine, erythrite, mannite, dulcitol, and sorbitol are not assimilated. The fact that it can utilize maltose, starch, and glycogen shows that it secretes the enzymes maltase, diastase and glycogenase.

5. Of the 17 nitrogenous substances tested as source of carbon only bacto-peptone, glutamic acid, histidine, tyrosine are utilized. Alanine, asparagine, aspartic acid, cystine and leucine are mediocre sources for the supply of carbon. Acetamide, cysteine hydrochloride, glycine, glycocoll, tryptophane, trihydroxy triethylamine, urea, and valine (each in 0.1 per cent concentration) cannot supply carbon necessary for the growth of the fungus.

6. The fungus is capable of utilizing nitrogen from alanine, ammonium nitrate, asparagine, aspartic acid, bacto-peptone, glutamic acid, histidine, tyrosine, valine, and, to a less extent, from leucine and acetamide. Cystine and cysteinehydrochloride are poor sources of nitrogen supply.

7. The fungus fails to grow in nutrient media where phosphate is absent. The minimum amount of K₂HPO₄ required for the growth of the fungus is 0.0001 gm. per litre.

8. The fungus is capable of hydrolysing peptone. Ammonification accompanies the reaction.

9. The fungus cannot hydrolyse fats.

10. The minimum, optimum, and maximum temperatures for the growth of the fungus are 4°C, 20°–27°C, and 32°C respectively.

INTRODUCTION

It is a matter of common knowledge that many fungi can grow in suitable synthetic liquid media in which usually a nitrogen source, a carbon source and certain essential minerals are present. Such fungi manufacture their own growth-promoting substances from the elementary ingredients of the nutrient media. There is another category of fungi which can grow in such synthetic media only when some growth-promoting substance is added from an extraneous source.

It has been pointed out by Farries and Bell (8) that *Nematospora gossypii* is unable to grow in synthetic media where the supply of nitrogen is in the form of hydrolysates of highly purified proteins except in the presence of small amount of an 'accessory factor' of the 'bios' type. Buston and Pramanik (4), Buston and Kashinathan (2), Buston, Kashinathan and Wylie (3), have also proved the necessity of the 'accessory factor' for the growth of *N. gossypii*. In this connection the works of Leonian (15), and Leonian and Lilly (16) on the nutrition of some fungi are also important. They have demonstrated that many of the fungi investigated by them fail to grow where thiamin or its intermediates are wanting. Schopfer (25, 26) reported that *Phycomyces blackesleeianus* Burgeff and some other fungi did not grow in a medium of mineral salts, pure dextrose, asparagine and water, but grew normally in the same medium to which a minute quantity of crystalline vitamin B₁ was added. Robbins and Kavanagh (20, 21) have confirmed Schopfer's report and have published their own valuable observations on the importance of vitamin B₁, and its intermediates on the growth of many fungi. Kögl and Fries (14) and Noecker (18) found vitamin B₁ to be essential for the growth of some *Basidiomycetes*. Recently Miss Hawkers (10) has found that *Melanospora destruens* does not grow on a synthetic medium consisting of glucose, KNO₃, MgSO₄ and KH₂PO₄ or with the addition of aneurin (vitamin B₁). She has made some other important observations also.

For the full literature on this subject the reader is referred to the works of the authors mentioned above and also to the articles of Bonner (1a) and Steinberg (27).

The present investigation deals with the synthesis of growth-promoting substances by *Saprolegnia delica* Coker; the effect of vitamin B₁ on its growth; its nutritional requirements; its power of hydrolysing peptone and fats, and its growth at various temperatures.

MATERIAL AND METHODS

The culture of *Saprolegnia delica* Coker was obtained from Central Bureau voor Schimmelcultures, Baarn, Holland.

The methods and technique employed in this work were the same as described by the senior author (23) in one of his previous papers.

All the experiments were carried on 25°C.

A. SYNTHESIS OF GROWTH-PROMOTING SUBSTANCES.

The four test fungi listed below and *Saprolegnia delica* Coker were maintained on potato dextrose agar and oat meal agar. A bit of mycelium was used as inoculum care being taken to avoid transferring any of the medium in which inoculum had grown.

A sterile solution (1 cc. containing 10 international units) of vitamin B₁ was prepared in distilled water. *

A nutrient medium (10 cc. in each tube) which will afterwards be referred to as medium M, containing 0.5 gm. each of K₂HPO₄, MgCl₂·6H₂O, 0.05 gm. of cystine, 2.0 gms. of NH₄NO₃, and 5 gms. of pure dextrose† per litre of distilled water was inoculated with the test fungi, *viz.*, *Phytophthora erythroseptica* Pethybridge (16), *Phycomyces blackesleeianus* Burgeff (+ strain), *Phytophthora fagopyri* Takimoto and *Mucor ramannianus* Moller (19), but there was no appreciable growth in any case indicating that the medium was free from the significant amount of thiamin, pyrimidine and thiazole.

A solution containing 10 units of vitamin B₁ was aseptically added to 10 cc. of the medium M in each tube, which was previously autoclaved. The tubes were then incubated for 72 hrs. at 30°C. The uncontaminated ones were inoculated with the test fungi. There was appreciable growth in all of them showing that the medium M contained all the necessary ingredients excepting vitamin B₁ for the growth of test fungi.

Experiment 1.—The medium M was poured in pyrex tubes (10 cc. in each), which were autoclaved and inoculated with *Saprolegnia delica*. The fungus grew well in the medium and was indefinitely transferable in it.

In the view of the fact that the fungus grows well in the medium M which is free from vitamin B₁, it seems that it is one of those fungi which do not require any growth-promoting substance from the extraneous source but manufactures it from the simple ingredients of the nutrient medium.

Experiment 2.—100 cc. of the medium M was poured in each of the 150 cc. Erlenmeyer pyrex flasks which were autoclaved. They were inoculated with *S. delica*, the standard incubation being 14 days in diffused light at room temperature (18–20°C). It was noticed that the fungus colony ramified in the medium and also reached its surface.

(a) The mycelium was removed aseptically, washed well in distilled water, which was changed several times. It was ground and added to 250 cc. of the medium M, which was then heated in the autoclave for 5 mts. at 5 pounds pressure; filtered sterile with the help of Seitz sterile filter and was poured in 20–25 pyrex

* A unit is 10⁻⁹. Mole of the compound.

† Dextrosol of Corn Product & Co., New York, U. S. A.

tubes, which were then incubated for 72 hrs. at 30°C. The uncontaminated ones were inoculated with the test fungi.

(b) An autoclaved nutrient medium containing 0.05 gm. each of K_2HPO_4 , $MgCl_2$, 0.005 gm. of cystin, 0.2 gm. NH_4NO_3 , 0.5 gm. of pure dextrose and 50 cc. of distilled water was added to 200 cc. of the medium from which the mycelium was removed as described in experiment 2a. It was then filtered sterile, poured in 20—25 pyrex tubes which were then incubated for 72 hrs. at 30°C. The uncontaminated tubes were inoculated with the test fungi.

It was found that the test fungi gave appreciable growth in both the experiments.

It has already been demonstrated that the medium M is free from the significant amount of thiamin, pyrimidine, and thiazole, and it is also a well-known fact that the test fungi require an accessory factor for their growth from an extraneous source in a nutrient medium. Since they grew readily in the media used in the above experiments, it can be assumed that the required accessory factor was added to the medium by the extract of the mycelium of *S. delica* (Exp. 2a.); that this organism synthesised its own growth-promoting substance from the simple ingredients of the medium M and that some of such synthesised substance was also given off by the mycelium into the medium M. (Exp. 2b.)

B. EFFECT OF VITAMIN B_1 ON GROWTH

10 cc. of a nutrient medium containing 0.1 gm. each of K_2HPO_4 , $MgCl_2 \cdot 6H_2O$, 0.5 gm. cystine, 0.8 gm. NH_4NO_3 , 1.0 gm. of dextrose and 1000 cc. of distilled water was poured in tubes.

(a) *Liquid medium.*

A sterile solution of vitamin B_1 in different concentrations (10 international units and 2 international units) was added to each tube containing the nutrient medium, before and after autoclaving.

The tubes in which vitamin B_1 was added after autoclaving were incubated for 72 hours at 30°C. The uncontaminated tubes were inoculated with the ball of the aerial mycelium of the fungus growing on oatmeal agar, care being taken to avoid including any of the agar of the stock cultures with the inoculum. In each case the inoculum was allowed to sink down in the nutrient medium.

Rise of the colonies within the tubes was measured on the fifth and seventh day.

TABLE I

Relative height (in cms) to which the fungus rises in the nutrient medium containing vitamin B₁.

Period of Growth.	Control. Nutrient medium alone.	2 Units of vitamin B ₁ added.		10 Units of vitamin B ₁ added.	
		Before autoclaving.	After autoclaving.	Before autoclaving.	After autoclaving.
4 days.	2.9	2.9	2.8	3.0	2.85
6 days.	3.9	3.9	4.0	4.0	4.0

(b) *Solid medium.*

To the nutrient liquid medium 2 per cent Difco bacto-agar was added and the experiments described above were repeated. Equal quantities of the medium were poured in Petri dishes (8 × 1.5 cm). These were inoculated with the fungus growing on the nutrient medium to which 2 per cent. Difco bacto-agar was added, the inoculum, in each case, being of the same size and age.

Growth of the colonies was measured on the third and fourth day.

TABLE II

Relative diametric growth (in cms.) of the fungus colony in nutrient medium + 2 per cent Difco bacto agar + Vitamin B₁.

Period of growth.	Control. Nutrient medium alone.	2 units of vitamin B ₁ added.		10 units of vitamin B ₁ added.	
		Before auto-claving.	After auto-claving.	Before auto-claving.	After auto-claving.
48 hrs. ...	3.7	3.97	3.85	3.95	3.95
72 hrs. ...	6.3	6.5	6.3	6.5	6.5

The results summarized in tables I and II show that there is no appreciable increase in the growth of the fungus in the presence of vitamin B₁ (2 or 10 international units per 10 cc. of the nutrient medium) and that the addition of vitamin B₁ neither accelerates nor retards the growth of the fungus. It is, therefore, one of those fungi which are not affected in their growth by the addition of vitamin B₁, *e.g.*, *Agaricus campestris*, *Absidia glauca*, *Basidiobolus rananum*, *Cunninghamella* spp.; *Mucor circinnelloides* (20).

C. SULPHUR REQUIREMENTS

Basal medium = 0.1 gm. each of K_2HPO_4 , $MgCl_2 \cdot 6H_2O$, 1.0 gm. of NH_4NO_3 , 2.0 gms. of dextrose and 1000 cc. of distilled water.

Medium for the source of inoculum = Basal medium + Difco bacto agar.

To the basal medium K_2SO_4 and Na_2SO_4 were added singly in 0.01 and 0.05 per cent concentrations and cystine in 0.05 per cent concentration. The media were inoculated with *S. delica* but there was no growth in the basal medium and the media containing K_2SO_4 and Na_2SO_4 , while the fungus grew well in the medium containing cystine and was transferable in it.

The results show that the organism requires sulphur for its growth and that it should be in the form of unoxidised sulphur such as cystine. Sulphates are not assimilated. Results reported by Leonian and Lilly (16) also indicate that organic sulphur (cystine) is necessary for the growth of *Saprolegnia mixta*, *S. parasitica*, *Achlya conspicua*, *Isoachlya monolifera*, and *Aphanomyces cantostylus*. Volkonsky (28, 29, 30) also got similar results with some members of the family *Saprolegniaceae*.

Various concentrations of cystine (0.025, 0.02, 0.01, 0.005, 0.0025, 0.002, 0.0015, 0.001, 0.0005, 0.00025, 0.0001 gms. per litre) in the basal medium were tried. The fungal growth decreased with the decrease in amount of cystine, the minimum amount necessary for its appreciable growth being 0.0005 gm. per litre.

D. CARBON REQUIREMENTS

Basal medium = 0.1 gm. each of K_2HPO_4 ; $MgCl_2 \cdot 6H_2O$; 0.025 gm. of cystine, 0.8 gm. of NH_4NO_3 and 1000 cc. distilled water.

Medium for the source of inoculum = Basal medium + Difco bacto-agar.

Series I.—Various carbon containing substances (15 carbohydrates and 5 alcohols) in 0.1 per cent concentration were added singly to the basal medium before autoclaving. The utilization of the carbon was indicated by the growth of the fungus in the nutrient media. Of all the substances inoculated with *S. delica*, only dextrose, levulose, maltose, dextrin, glycogen and soluble starch were utilized by it, and were most favourable for growth and acidification. Arabinose, galactose, mannose, rhamnose, xylose, lactose, sucrose, raffinose, inulin, glycerine, erythrite, dulcitol, mannitol, and sorbitol in 0.1 per cent. concentration did not provide carbon for the growth of the fungus.

The fact that the fungus can utilise maltose, starch and glycogen gives an indirect proof that the fungus secretes the enzymes maltase, diastase and glycogenase. Where the carbohydrates were utilized, the nutrient media after the growth of the fungus became more acidic, confirming the well established fact that fungi generally produce various organic acids as product of their metabolism in the presence of carbohydrates.

In the control, which was lacking in the source of carbon, there was no growth of the fungus.

Series II.—In order to avoid the risk of the carbohydrates being hydrolysed in the presence of the basal medium during autoclaving, the solution of the carbohydrate to be tested and that of the basal medium were autoclaved separately and were mixed together after cooling. The tubes were then incubated for 72 hours at 30°C. The uncontaminated ones were inoculated with *S. delica*. The results were practically the same as already described above.

Series III.—Some nitrogenous substances in 0.1 per cent concentration were also tried as source of carbon in the nutrient medium. Of the 17 nitrogenous substances tested, only bacto-peptone, glutamic acid, histidine, and tyrosine were found to be good sources of carbon, while alanine, asparagine, asparatic acid, cystine, and leucine were mediocre sources. Acetamide, cysteine hydrochloride, glycine, glyecoll, tryptophine, trihydroxy triethylamine, urea and valine in 0.1 per cent concentration could not supply carbon necessary for the growth of the fungus.

The results obtained are in general agreement with those of Volkonsky (28, 29, 30) who investigated the nutritional requirements of a number of fungi belonging to the family *Saprolegniaceae*. Like several species of *Pythium* (23), *Saprolegnia delica* Coker is incapable of utilizing arabinose, galactose, rhamnose, xylose, lactose, raffinose, inulin, erythrite, mannite, dulcite, sorbite, acetamide and urea as source of carbon.

Various concentrations of dextrose (0.1, 0.05, 0.01, 0.005 gm. per litre) were tried in the basal medium, which contained only 0.0015 gm. per litre of cystine. It was found that the fungus growth decreased with the decrease in the amount of dextrose, the minimum amount necessary for its appreciable growth being 0.05 gm per litre in the presence of 0.0015 gm. of cystine.

E. NITROGEN REQUIREMENTS

Basal medium = 0.1 gm. each of K_2HPO_4 , $Mg Cl_2 \cdot 6H_2O$, 0.025 gm. of cystine, 2.0 gms. of dextrose and 1000 cc. distilled water.

Medium for the source of inoculum = Basal medium + Difco bacto agar.

To the basal medium various nitrogen-containing substances were added singly in 0.1 per cent concentration. It was found that alanine, ammonium nitrate, asparagine, asparatic acid, Difco bacto-peptone, glutamic acid, histidine, tyrosine and valine were good sources of nitrogen; leucine and acetamide were mediocre; cystine and cysteine hydrochloride were poor sources while glyecoll, glycine, trihydroxy triethylamine and urea in 0.1 per cent concentration could not supply nitrogen to the organism.

Some of these results are at variance with those of Volkonsky (28, 31) who has reported that acetamide is not assimilated by *Saprolegnia dioica* while glycocoll and urea are utilised by the fungus, but are in agreement with those reported by Leonian and Lilly (16) and Saksena (23). *S. delica* is also one of those fungi (16, 23) which can manufacture their own amino acids from NH_4NO_3 or from a single favourable amino acid.

Various concentrations of ammonium nitrate (0.1, 0.05, 0.01, 0.005, 0.001 gm. per litre) were tried in the basal medium, which contained only 0.0015 gm. per litre of cystine. It was found that the fungus growth decreased with the decrease in the amount of ammonium nitrate, the minimum amount necessary for its appreciable growth being 0.005 gm. per litre in the presence of 0.0015 gm. of cystine.

F. PHOSPHORUS REQUIREMENTS

Basal medium = 0.1 gm of MgCl_2 , 0.015 gm. of cystine, 0.1 gms. of NH_4NO_3 , 2 gms. of glucose and 10.0 cc. distilled water.

Medium for the source of inoculum = Basal medium + Difco bacto agar.

To the basal medium was added K_2HPO_4 in 0.1 per cent concentrations. There was no growth in the basal medium while the fungus grew well in the medium containing K_2HPO_4 showing that a phosphate is necessary for the growth of the fungus.

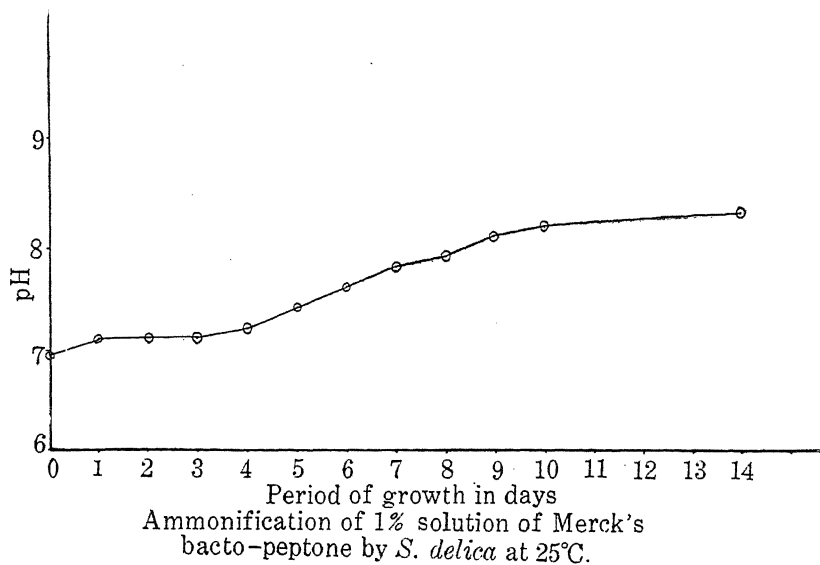
Various concentrations of K_2HPO_4 (0.01, 0.005, 0.001, 0.0005 gm. per litre.) in the basal medium containing only 0.0015 gm. of cystine were tried. It was found that the growth of the fungus decreased with the decrease in amount of K_2HPO_4 , the minimum amount of K_2HPO_4 as source of phosphorus required by the fungus being 0.001 gm. per litre.

G. HYDROLYSIS OF PEPTONE

Saprolegnia delica was grown in 150 cc. Erlenmeyer pyrex flasks each containing 100 cc. of 1 per cent solution of bacto-peptone (Merck). Stock cultures for inoculum were maintained on a medium containing 0.1 gm. of bacto-peptone, 1.8 gm. of Difco bacto-agar and 100 cc. of distilled water. The nutrient medium remained clear during the growth of the fungus. The pH determinations and qualitative tests with Nessler's reagent for ammonia were made at 24-hour intervals for 14 days (Graph I); at each determination the pH value and the test obtained were compared with those of the control. Graph I indicates that hydrolysis of 1 per cent solution of bacto-peptone was accompanied by an increase in alkalinity as is the case in several species of *Pythium* (22).

Qualitative tests made with Nessler's reagent indicated the presence of ammonia in traces after the end of the first day. The reaction became more intense as the

growth proceeded showing the presence of greater amount of ammonia. The hydrolysis of peptone is due to some protolytic enzyme which the fungus secretes during its growth.



Graph I

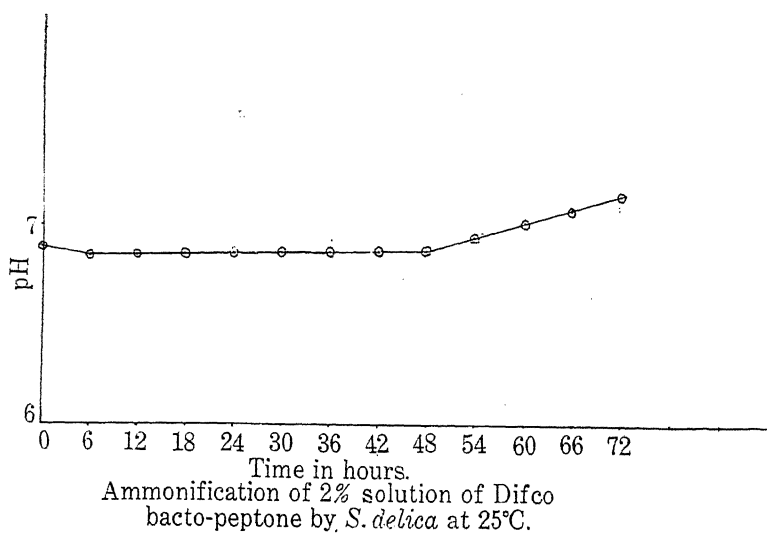
Recently, Wolf (32) has reported interesting results in the case of *Saprolegnia ferax* and *Achlya bisexualis*. He found that after inoculation, there was first a lag phase of 6 hours duration, followed by a slight drop in the pH of 2 per cent solution of Difco bacto-peptone, presumably due to the acidity of the mycelium. The initial pH of the solution was 7.1. At the end of 10 hours in the case of *S. ferax*, the pH of the solution became 6.9 approximately. A gradual rise then occurred and the pH of the solution became 7.8 at the end of 40 hours in both the cases, and subsequently there was no change. With *S. ferax* the rise to maximum pH occurred between 16 and 30 hours. These observations are not in accord with those obtained with *S. delica* in which case a gradual increase in alkalinity continued even upto the end of two weeks.

In order to study the changes in the pH range in early stages and to render the results comparable, Wolf's procedure was adopted in the following experiment.

S. delica was inoculated on 50 cc. of two per cent solution of Difco bacto-peptone contained in 150 cc. Erlenmeyer flasks*. The pH determinations and qualitative tests with Nessler's reagent for ammonia were made at 6 hour intervals for 72 hours; at each determination the pH value and test obtained were compared

* one flask for each determination,

with those of the control. The medium for source of inoculum consisted of 0.1 gm. of Difco bacto-peptone, 2.0 gms. of Difco bacto agar and 100 cc. of distilled water.



Graph II.

Graph II shows that the fall in the pH of the solution was negligible becoming 6.85 at the end of 6 hours, followed by a "lag phase" upto 48 hours. After this the pH began to rise. These results are at variance with those reported by Wolf (32) in the case of *Saprolegnia ferax* and *Achlya bisexualis* and are in general agreement with those of the senior author in case of some species of *Pythium* (23).

Klebs (12), Schmidt (24), Volkonsky (28, 29), Guilliermond and Obaton (9), and Wolf (32) have reported that peptone hydrolysis is accompanied by the production of ammonia. Butkewitsch (5) has shown that *Aspergillus niger* hydrolyses the peptone, but provokes the formation of enormous quantity of ammonia which does not alkalinise the medium because it is neutralized by the formation of oxalic acid. Waksman and Joffe (31) in case of *Actinomyces* have reported that the medium containing peptone becomes generally alkaline and these results were confirmed by Nässlung and Dernby (17). Klotz (13) in case of *Diplodia natalensis*, *Phoma betae* and *Sphaeropsis malorum* has reported that the medium is alkalinised and pH rises upto about 8.4. Bach (1) in his studies on *Aspergillus repens* de Bary has reported similar results.

H. HYDROLYSIS OF FATS

10 c.c. of the nutrient medium, consisting of 0.1 gm. each of K_2HPO_4 and $MgCl_2 \cdot 6H_2O$, 0.05 gm. of cystine, 1.0 gm. each of NH_4NO_3 and dextrose, 20 gms,

of agar and 1000 cc. of distilled water, was poured in pyrex tubes. To each tube was added 1 cc. butter fat, obtained by melting the butter, causing the butter-fat to separate from the curd. The tubes were then autoclaved, cooled to 40°C, shaken vigorously and poured in sterilised Petri dishes of equal size (10×1½ cm) Petri dishes containing the nutrient medium alone served as control.

The plates were incubated for three days at 30 C. The uncontaminated ones were inoculated with *Saprolegnia delica*, growing on a medium containing 0.1 per cent bacto peptone and 2 per cent agar. Diametric spread of the colony was measured on the second, third and fourth day. (Table III).

TABLE III

Relative diametric growth of the fungus (in cms) on the nutrient medium containing butter fat.

At the end of	Nutrient medium	Nutrient medium + fat
1 day	2.9	2.9
2 days	5.0	5.2
3 days	7.15	7.15

The results summarised in Table III show that the growth was nearly the same on both the media. Had there been secretion of lipase by the fungus to hydrolyse the fat there would have been more rapid growth on the medium containing fat.

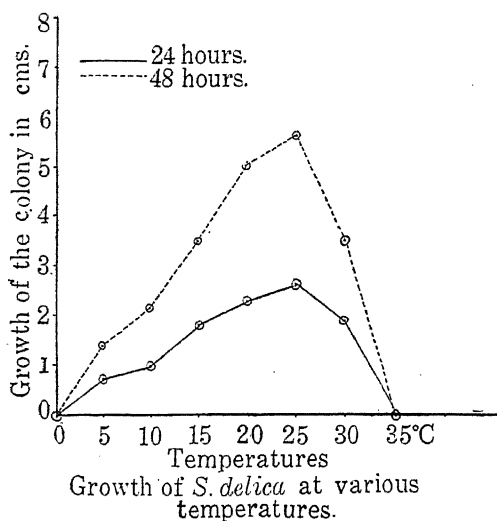
The results obtained above are in agreement with those of Emoto (7) who has reported that lipase was not produced by *S. Tokugawana* Emoto and an unidentified sterile species of *Achlya*, but they are at variance with the observations of Kanouse and Humphery (11) who have reported that *Pythium afertile* K and H showed an excellent growth on a medium containing corn oil or castor oil as the only organic nutrient and also with the observation of Wolf (32) who has found that both *Saprolegnia ferax* and *Achlya bisexualis* are capable of hydrolysing fats.

I. GROWTH OF *SAPROLEGNIA DELICA* AT VARIOUS TEMPERATURES

The fungus was grown on dextrose peptone agar (0.1 gm. dextrose + 0.1 gm. Difco bacto-peptone + 2 gms. Difco agar + 100 c.c. of distilled water) at various temperatures. (0°, 5°, 10°, 15°, 20°, 25°, 30°, 35°C.) Inoculum of the same age and size (4×4 mm.) was taken from the margin of the growing colony maintained on the same medium. In each case the Petri dishes were kept for 2 hours, before inoculation, at the temperature on which an experiment was carried on, to remove the lag effect

The diametric growth of the colony was measured on the second and third day. (Graph III).

It was found that at 0°C there was no growth either at the end of 24 or 48 hours. The graph then shows a gradual rise attaining the maximum height at 25°C . The optimum temperature favourable for the growth of the fungus is between $20^{\circ}-27^{\circ}\text{C}$. This is followed by a rapid drop reaching the minimum at 35°C .



Graph III.

In order to know the exact temperature at which growth starts or stops determinations were made at $1^{\circ}, 2^{\circ}, 3^{\circ}, 4^{\circ}, 5^{\circ}$, and $31^{\circ}, 32^{\circ}, 33^{\circ}, 34^{\circ}, 35^{\circ}\text{C}$. (Table IV)

TABLE IV

Relative growth of the fungus (in cms.) at various temperatures after 24 hrs. and 48 hrs.

Temperature	Growth of colony after 24 hours	Growth of colony after 48 hours.
0°C	No growth	No growth
1°C	No growth	No growth
2°C	No growth	No growth
3°C	No growth	No growth
4°C	No growth	0.6
5°C	0.7	1.4
31°C	1.0	1.9
32°C	No growth	No growth
33°C	No growth	No growth
34°C	No growth	No growth
35°C	No growth	No growth

The results summarised in Table IV show that there was no growth upto 3°C at the end of either 24 or 48 hours. At 4°C some growth (0.6 cm.) was observed at the end of 48 hours. Appreciable growth (0.7 cm.) was noted at 5°C at the end of 24 hours. At 32°, 33°, 34°, 35°C also there was no growth either at the end of 24 or 48 hours, while at 31°C the fungus showed growth (1.0 cm.) even at the end of 24 hours

Thermal death points:—

The fungus was grown at 3°, 2°, 1°, 0°, 32°, 33°C on the same nutrient medium as was used for determining the growth of the fungus at various temperatures. The Petri dishes were removed from the inside of the incubator to room temperature after 6-hour intervals for a period of 48 hours. It was observed that the fungus began to grow when kept at room temperature after it was removed from the incubator maintained at 3°, 2°, 1°, 0°, and 32°C.

These results show that the fungus can tolerate temperatures 0° and 32°C for at least 48 hours, and it does not die but becomes dormant, regaining its vitality on getting a suitable temperature. At 33°C the fungus made no growth when it was removed from the incubator even after 6 hours showing that it could not tolerate the temperature and that it died. The high thermal death point for the fungus lies between 32° and 33°C.

Duff (6) in a species of *Saprolegnia* has reported 35°C, 1°C, 20° to 25°C to be the maximum, minimum and optimum temperatures respectively.

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PHYSIOLOGICAL STUDIES ON THE WHEAT PLANT (CONTD.) IV.
THE EFFECT OF MANURES ON THE HYDROGEN-ION
CONCENTRATION OF THE ROOTS AND LEAVES OF *TRITICUM*
VULGARE AND OF THE SOIL AND THE NITRATE
FLUCTUATIONS IN SOILS

BY SHRI RANJAN AND KRISHNA GOPAL RAJIVANSHI

BOTANY DEPARTMENT, ALLAHABAD UNIVERSITY

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SUMMARY

The hydrogen-ion concentration of normal soils, during the growth of wheat, shows that it is high at first then it falls and again increases. The fall in the pH or in other words the increase in acidity may be due to the removal of the metallic cations from the soils when the plants are actively growing. The cations, thus removed, get replaced by hydrogen-ions and the soil then tends to become acidic. When the plants are fully grown less of the metallic-ions are removed from the soils which then shows a relative increase of alkalinity.

An estimation of nitrates at various depths of soils go to show that the nitrate content rapidly diminishes as one proceeds downwards in the soil, except in the case of compost soil where, upto a depth of $1\frac{1}{2}$ feet, the nitrate content increases and thereafter it decreases. This great increase, in the compost bed, at a depth of $1-1\frac{1}{2}$ feet may be responsible for higher yield as compared to the yield in the molassed and sub-soil beds.

In the previous papers on wheat from this laboratory* the investigations were undertaken with a view to establish correlations, if any, with amino and total nitrogen and the yield and with chlorophyll and carbohydrate contents.

In this paper an attempt has been made to establish correlations with hydrogen-ion concentrations of the substratum (soil) and roots and leaves of wheat plants at different ages of the plant and with nitrate fluctuations of the soil

The present work is divided into 2 sections. The first deals with the determinations of pH values of the soils, the cell sap of roots and leaves of the wheat plant. The observations were recorded at regular intervals, as far as possible, along with other research workers working on the same plant with different objectives.

The second section deals with the amount of nitrates in the soils at different depths.

* Physiological studies on the wheat plant. Part I. The effect of manures on the total nitrogen and amino acid nitrogen in *Triticum vulgare* and soil—by S. N. Bhattacharya and Shri Ranjan.

Physiological studies on the wheat plant. Part III. The chlorophyll and carbohydrate contents of *Triticum Vulgare* in relation to manures —by Gopi Narain Dikshit and Shri Ranjan.

METHOD

Determination of Hydrogen-ion concentration.—For the determination of Hydrogen-ion concentrations the potentiometric method was employed. The apparatus was standardized before using. The barometric pressure was noted for each observation and the observed electromotive force was corrected for pressure from the Table given by Clark⁴ (1920).

The roots of the plants, taken out for experiments, were thoroughly washed with tap water, particular care was taken that nothing else remained attached to the roots. For the extraction of cell-sap from the roots and leaves, the material was weighed, crushed in a glass mortar, and made to a known volume.

For soil solution, the soil was weighed and dissolved in a known amount of water. This proportion was in every case maintained the same. The solution thus prepared was stirred and centrifuged. The hydrogen-ion concentration of the cell-sap and the soil solution was then determined.

It was interesting to note that the same solution when kept for the next day, showed a decrease in pH value, thereby showing a tendency to go towards the acidic side. In order to avoid this, the cells were made chemically inactive before being crushed by the method of Ingalls and Shive⁵ (1931).

Nitrates—For the estimation of nitrates Devardas Alloy Reduction Method was tried, but being too tedious was given up. Phenol-disulphonic acid method was also tried but because of the difficulties in its preparation this method was also given up. Finally the diphenylamine method was adopted.

The reagent was prepared by dissolving 0.025 gm. of diphenylamine in 425 c.c. of extra pure conc. H_2SO_4 and this was diluted to 500 c.c. with distilled water.

Tests were conducted by mixing 0.05 c.c. of Conc. HCl. with 2 c.c. of the solution to be examined together with 10 c.c. of the above reagent. After 10 minutes the colour produced was compared with the standard KNO_3 solution prepared in the same manner. Differences of 0.0001 per cent in the amount of the NO_3 present in a given solution could easily be detected by this method.

SECTION I

Hydrogen-ion concentration.—Arrhenius¹ studied the growth of crop plants under media of varying hydrogen-ion concentrations and concluded that acidity or alkalinity of the substratum has a distinct influence on the growth response of plants. Hoagland⁷, on the other hand, has shown that slight acidity is not injurious to crop plants, but pH values of 9 and above are injurious. Atkins² at Pusa found that there was a wide divergence of pH concentrations between the stems, roots and leaves of the same plants. Smith and Quirk working on the different parts of *Begonia lucerna* found that while the leaves have a pH ranging from 0.9 to 1.36 the top of the shoots

have 1.22 to 2.23 and the base of the shoots have a pH range of 3.30 to 3.42. Miyaki and Masashi found in the case of oats and flax that either phosphoric acid or potassium, applied to the soil, tends to raise the pH concentration of the cell sap while nitrogen tends to lower it. Truog and Meacham⁹ grew a few plants on limed and unlimed soils. In 12 out of 16 cases the plant juices were more acidic on the unlimed soils than on the limed ones. Dastur and Kalyani⁵ worked on both the soil and the plant juices of rice and are of opinion that pH values of $(\text{NH}_4)_2 \text{SO}_4$ beds are the lowest and those of the NaNO_3 beds are the highest.

Hydrogen-ion concentration of soils.—For our experiments we took soil samples, from the wheat beds of the botanical garden, as follows:—

- (a) *Subsoil bed.* In this bed all the surface soil was removed upto a depth of 2 ft. and subsoil from a neighbouring bed was thrown in.
- (b) *Molasses bed.* The soil in this bed was the same as the above subsoil, but molasses was added into this according to the specifications of Dhar⁶.
- (c) *Compost bed.* This consisted of farmyard manure mixed in ordinary surface soil in the usual proportion.
- (d) *Surface soil bed.* This contained only the ordinary surface soil of the garden.

Fig. I (A, B and C) gives the pH values of the various soils, and the roots and leaves of plants growing in them. The pH of the soils before manuring was high, being definitely on the alkaline side. But when molasses and farmyard manure were

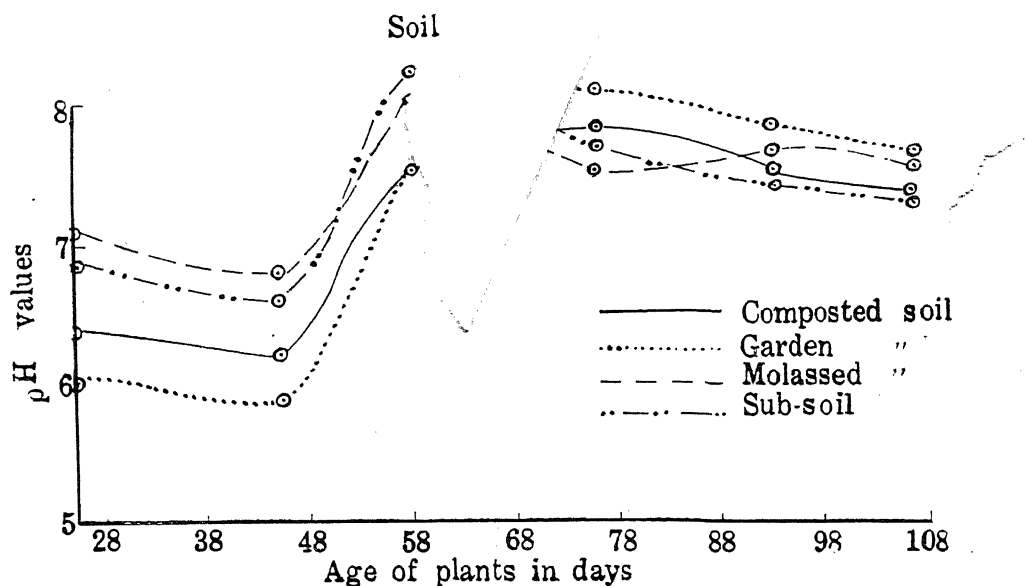


Fig. IA

added to the subsoil and surface soil respectively there was a considerable fall in the pH values. This agrees with the result of Dhar and his co-workers.

In all the four beds wheat seedlings were planted at a time when the pH of these beds ranged between 5.3 to 6.5. This earlier part of the curve is not indicated in Fig. 1A. The pH of these soils now gradually increased for about 8 days, thereafter it fell off to rise again showing a typical \sim shaped curve (Fig. 1A). The peak was attained in about 2 months, when the plants in these beds began to show ear formation. These curves also show that whereas the pH at the initial stages in the cases of subsoil and molasses bed is higher than the compost and the surface soil control beds, it tends to equalise in about 2 months. These changes in the pH values of the soils at different periods may be explained if we assume that the soil particles can partially remove any cation from solutions which get loosely attached to the soils. We may then think of the soil particle with its cations as a salt in which the colloidal bodies of the particle constitute the anion. Wiegner has shown that hydrogen ions are also associated with clay particles. In the case of the 'saturated' soils, the whole of the absorptive power of the soil is satisfied with metallic cations, but when hydrogen ions are among the cations the soil is unsaturated. If to such a soil with its hydrogen ions a neutral salt solution is applied, the solution becomes acid,

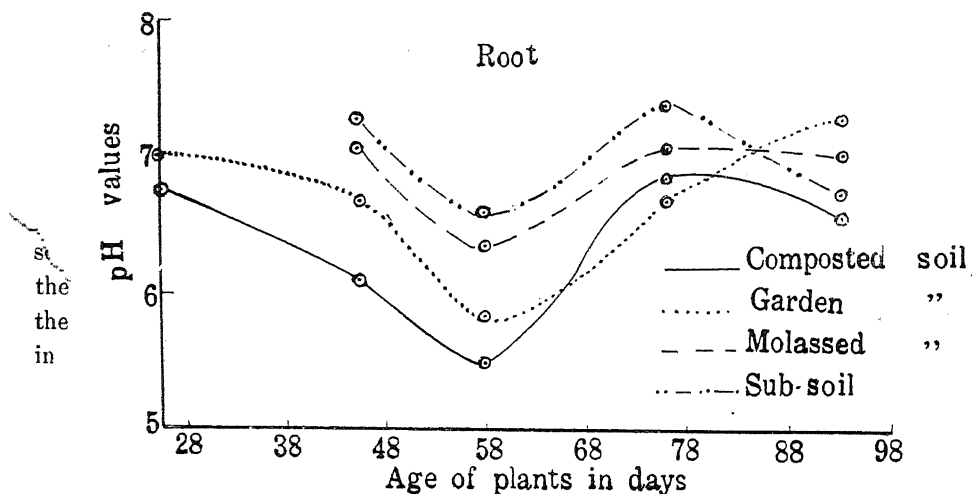


Fig. 1B

for the simple reason that the ionic exchange occur whereby hydrogen ions come into solution. A full 'saturated' soil which has the absorptive power fully satisfied with metallic cation will be an alkaline system comparable to the salt of a strong base and a weak acid, *e.g.*, sodium phosphate. Thus during plant growth if the roots removed the metallic cations from the soils, and these get replaced by hydrogen

ions, then the soil will tend to become acidic. Thus during the period that the plant is rapidly growing, *i.e.*, for the first one and a half months, the cations are being rapidly removed and the soil pH shows a trough in the graph, thereafter, the removal of the metallic cations from the soils is slowed down and the pH graph shows the crest

Figures IB and IC show the pH curvatures of the roots and leaves respectively.

In comparing these graphs with Fig IA one notices that the troughs of IA correspond with the crests of IB and IC while the crests of IA corresponds to the troughs of IB and IC. This apparent conflicting results may be explained on the assumption that when there is a maximum absorption of the metallic ions by the soil particles, the soils show the trough and at that particular period the roots and consequently the leaves not having absorbed these ions, in sufficient quantity—the roots being poorly developed in the early stages of growth—show a crest. Later on, say after about a month by the absorption of the metallic ions by the roots, the soils become acidic—by the replacement of the metallic ions of the soil particle by hydrogen ions—and the roots relatively alkaline. In about 2 months time the wheat plant, now fully matured, sets ears and the absorption of ions by the roots decrease. This then brings about the crest (Fig. IA) in the soil pH. At the time of the formation of the ears the metallic ions now travel from the cells of the roots and leaves and migrate towards the ears, thus causing troughs in the pH of these organs.

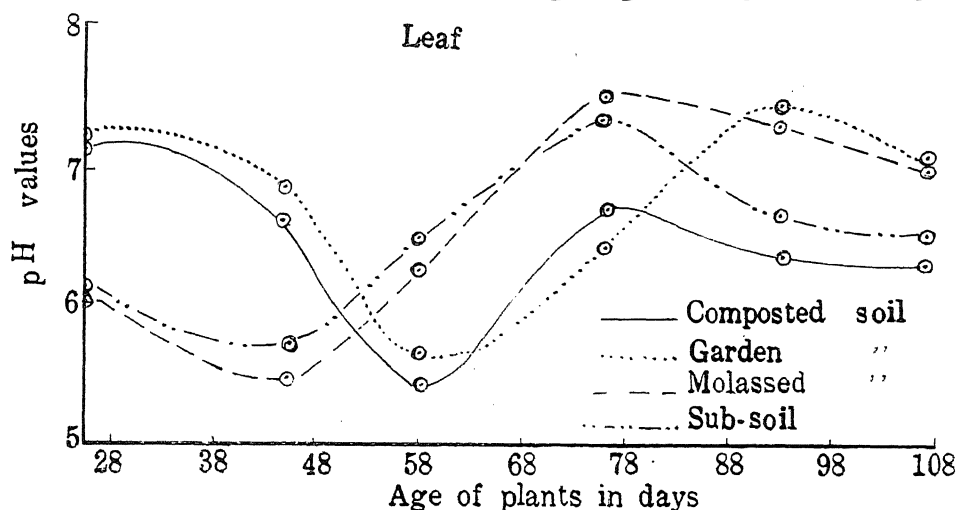


Fig. IC

Figures IA, IB and IC also show that the magnitude of the change in the pH values of different beds corresponds with the magnitude of the change in the pH of the roots growing in these beds. For instance, the pH of the molassed soil and subsoil is higher than those of the compost and control soils; so also one finds

the pH of the roots growing in the subsoil and molassed bed higher than the pH of roots growing in the compost and control beds.

Bhattacharya and Ranjan⁸ have shown that when the wheat plants are 30—40 days old the amino-acid nitrogen and total nitrogen are the lowest. We find the pH in the roots and leaves are the highest. Then again between 45—55 days the amino-acid nitrogen and total nitrogen increases while correspondingly the pH decreases. Thus it is possible that amino-acids, though weak acids, may be effecting the acidity of the cell saps.

SECTION II

Nitrates of the soil.—The amount of nitrogen in the plant is to a large extent determined by the concentration of this element in the soil. Gurney and Sewell

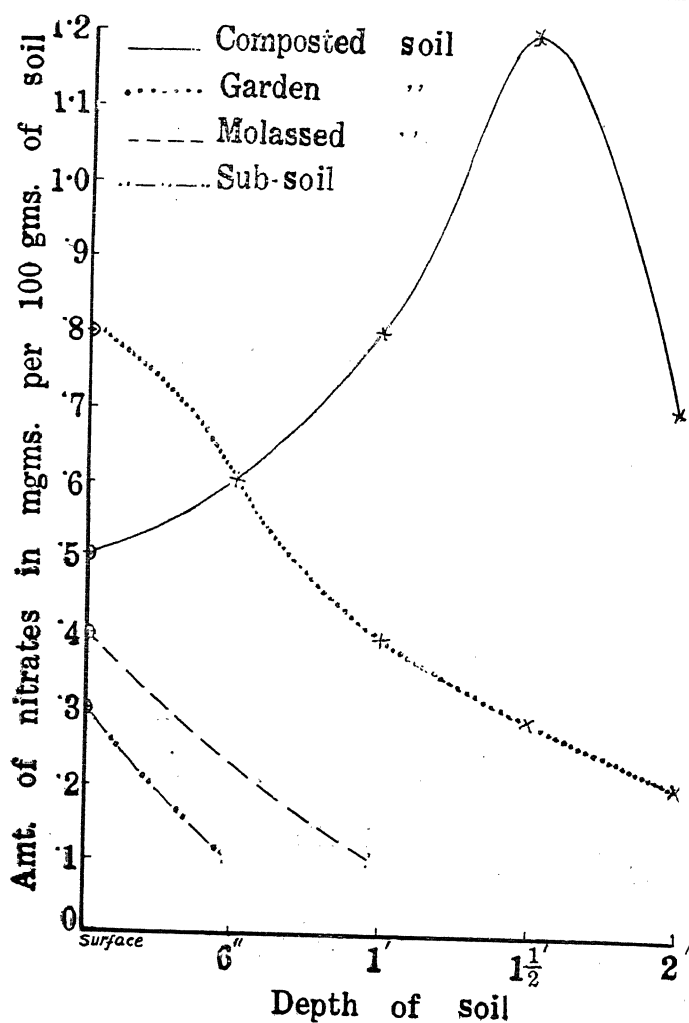


Fig. II

studying the 'Nitrogen spots' in the wheat fields of Kansas, reported that these spots are characterized by higher nitrogen content, than that of the adjoining soil, and the plants growing in these spots have a higher nitrogen content. Richardson and Gurney found that nitrogen accumulated in fallow soil, and this was an important factor in accounting for the heavy yield from fallow as compared to stubble land.

The nitrate content of the four types of soil beds at various depths have been represented by Fig. II for December and by Fig. III for January.

A study of Fig. II shows that the nitrate content of the surface soil of the subsoil bed is as low as .3 while that of the control is as high as .8. The molassed bed shows the nitrate content to be .4. The amount of nitrate falls, in all cases except the compost bed, as we proceed to lower depths. In the case of the compost bed there is an actual increase of nitrates as one proceeds to lower depths and at $1\frac{1}{2}'$ depth

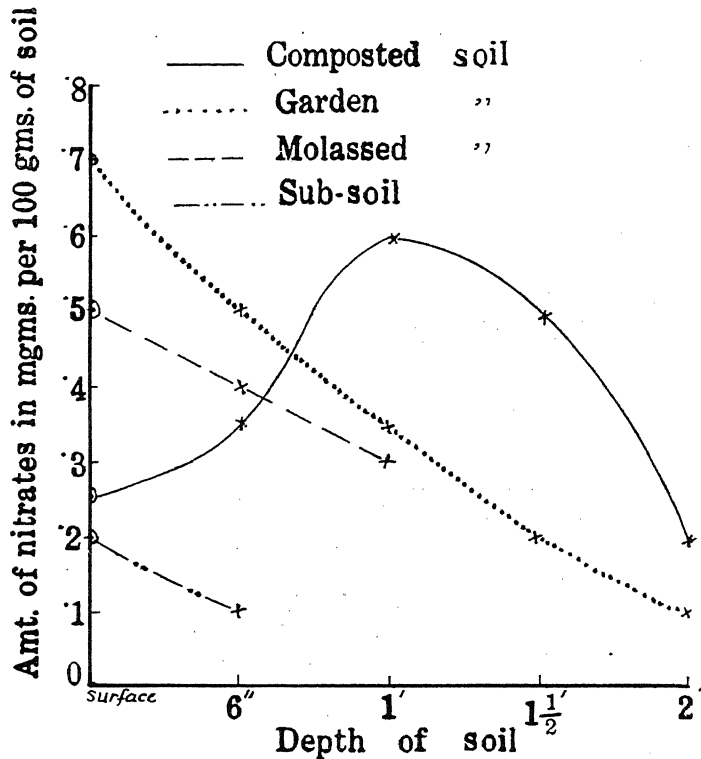


Fig. III

the nitrate content is highest, thereafter there is again a fall. This increase of nitrates at such low depths may be due to the production of nitrates by the activities of soil bacteria acting upon the organic nitrogenous material in the farmyard

manures. The nitrates so formed descend to lower depths. This may also account for the larger yield of the crop, because the roots of the wheat plants ramify profusely at this depth. The yield of grains from plants growing in the 4 beds is given below.

Plot	Yield of grain in gms. per 4 plants.
Compost	7.30
Surface soil	7.13
Molassed subsoil	6.45
Subsoil	4.28

The January figures for nitrates, shown in Figure III, follow in general the same type of course as the December one (Fig. II), with this difference that the values of nitrates in all the beds except the molassed bed is lower than the December values.

Here again, as one goes down the soil, nitrate increases in the compost bed and after a depth of 1 foot again starts decreasing. The surface soil control and the subsoil beds show a typical fall with increasing depth. On the other hand though the molassed soil bed does show a fall, in its nitrate content, with increasing depth, nevertheless the nitrogen content is appreciably higher in January than in December. This, as has been shown in a previous paper of this series, may be due to the action of 2 factors, *viz.*, (i) bacterial activity causing breakdown of the higher nitrogenous matter in the molasses and (ii) the Photochemical formation of nitrates in the molassed beds, acting singly or jointly.

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